Synthetic efforts towards 6H - pyrido [4,3-b] carbazole derivatives.

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SYNTHETIC EFFORTS TOWARDS 6H-PYRIDO [4,3-b] CARBAZOLE DERIVATIVES

Submitted by DAVID WATKINS
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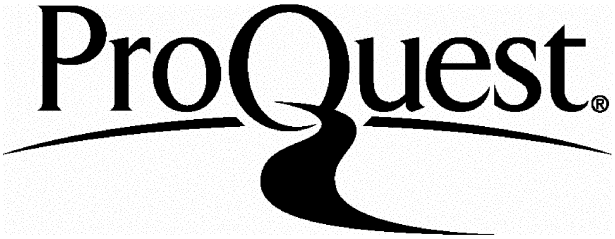

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Protocol demands that I should thank my friends of past and present in the department, although their contribution is an abstract one: the work was completed despite their efforts. Seriously, though, many hours of mirth have helped to punctuate life in the laboratory to good effect.

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Finally, I would like to thank my parents for their support and encouragement throughout what has proved to be a protracted academic career.

SUMMARY

The work outlined in this thesis was conducted between October 1977 and September 1980 and is primarily concerned with the synthesis of ellipticine derivatives, which materials show promise as potential antitumour agents.

At the time work commenced there was a need to develop an improved method of preparation for the starting material which is fundamental to the ellipticine synthesis favoured by workers at Bath. This synthesis is recognised as a versatile route to a variety of derivatives, but so far an efficient preparative method for the starting material - a pyridylethyl indole - has not been found. Alternative strategies are discussed here.

The next section deals with the preparation of some new derivatives of ellipticine substituted in the 5-position with a variety of functional groups, and highlights some of the drawbacks of the known method which had previously not been encountered.

The ^{13}C nuclear magnetic resonance spectrum of ellipticine is discussed at some length in relation to the apparent regio-specificity of the reaction in which it is formed, and spectral data are compared with those of other workers. As yet, the assignment of ^{13}C chemical shift values remains fairly empirical, but from comparison with model compounds a new interpretation of the ellipticine spectrum has been proposed.

Finally, attempts to prepare 6-methyl-and 10-hydroxy-derivatives of ellipticine are described, the first of these involving a new synthetic route and alternative methods of preparation of 4-acetylpyridines.

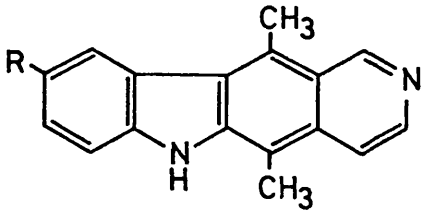
CONTENTS

INTRODUCTION

INTRODUCTION

The alkaloids ellipticine (1a, R=H) and 9-methoxyellipticine(1b,R=OCH_3) are 6H-pyrido [4,3-b] carbazoles which were first isolated by Goodwin, Smith and Horning[1] from the leaves of the plant Ochrosia elliptica Labill. It is now known that these natural products and structurally-related compounds are to be found in other plants, in particular from the Aspidosperma, Ochrosia and Tabernaemontana genera of the family Apocyanaceae[2].

These alkaloids and some of their derivatives are of special interest because they show promise as potential anti-cancer drugs[3]. This has stimulated a considerable effort to synthesise new analogues and derivatives of 6H-pyrido[4,3-b] carbazoles for pharmacological testing and evaluation.



1a, R=H
1b, R=OCH_3
1c, R=NH_2

Cancer therapy:

Despite considerable efforts during recent years it remains sadly true that cancer is still one of the major causes of death. During 1975, in England and Wales malignant diseases accounted for some twenty-one per cent of all deaths. In the same period mortality due to cardiovascular diseases was nearly forty per cent, but since the beginning of the century the death rate due to cancer has been steadily increasing. These figures reflect the effective-

ness with which other forms of fatal disease have been combatted with advances in medical science, but as yet the treatment of cancer has seen no dramatic breakthrough.

Cancer treatment is complicated by the fact that it is not a single disease. The word cancer is an all-embracing term which describes a number of disease processes affecting multicellular organisms. The common characteristic of these processes is the apparently uncontrolled multiplication and spread of abnormal forms of cells within the organisms.

In essence three therapeutic weapons are available, namely surgery, radiotherapy and chemotherapy, although much excitement has been generated recently in the field of immunotherapy. The fact that surgery still holds pride of place is embarrassing to some who feel that this is an admission of failure. Nevertheless, in many manifestations of the disease, excision of the malignant growth offers the best, and often the only, hope of a cure. To be most effective surgery should be carried out before metastases, or secondary growths, occur and for this reason early diagnosis is advantageous. One of the major arguments against surgery is the belief that the operation itself may be responsible for spreading the disease to distant parts of the body by dislodging malignant cells and permitting them to be transported through the body by means of the circulatory system. In particular this may give rise to metastases in organs which have an especially rich blood supply, such as the liver, kidneys or lungs.

Radiotherapy is still the second most widely used form of cancer treatment and has been successfully employed since the early years of this century. The tumour and its immediate surroundings are exposed to ionising radiation which may be generated by a variety of sources. The radiation kills both malignant and

normal cells, but its lethal effect is seen only by those cells which are undergoing division. It is not necessarily true to say that malignant cells divide more frequently than normal cells. Certain types, such as bone marrow cells which manufacture the cells of the blood, and those which line the walls of the digestive tract are exceptional because they divide frequently. In general, however, normal cells will only divide when called upon to replace others which have died, or during growth processes. Cancer cells have somehow lost the ability to recognise that further division is unnecessary and it is this failure which leads to tumour growth. Radiotherapy has no special selective effect upon malignant cells, and consequently there is always associated damage to healthy tissue in the vicinity of the tumour. Fortunately healthy cells recover more quickly from radiation doses which are near-lethal and are able to repair damage during intermissions between repeated periods of radiotherapy. Cancer cells which are damaged but not killed may then be knocked out by a subsequent exposure before they are able to carry out the necessary repair work.

Chemotherapy is the latest addition to the effective forms of treatment of malignant disease but it suffers from the same limitations as radiotherapy in that healthy and cancer cells alike are both damaged by the treatment. It is fortunate, then, that healthy cells are able to recover, at least in part, from a near-lethal dose of a chemotherapeutic agent. Despite these drawbacks, much hope for the future rests with chemotherapy, since it seems certain that some essential differences exist between the vital processes of the cancer cell compared with those of the healthy cell. When these differences are recognised the long-awaited specific anti-cancer drug becomes a real possibility, as it should

be feasible to design a drug which disrupts the vital processes of cancer cells without disturbing healthy ones.

The cell cycle in cancer chemotherapy:

One of the major goals of current work in cancer therapy is to determine the best possible scheme of treatment for a given drug, or combination thereof, with respect to dose levels and intervals between courses. The intention is to kill disseminated cancer cells at a faster rate than they can be replaced, for long enough to ensure that their number is reduced to zero, and above all without over-dosing the host.

Awareness of the concept of the cell cycle[5] has done much to clarify the mechanism of action of certain chemotherapeutic agents and this in turn has led to improved design of treatment regimens.

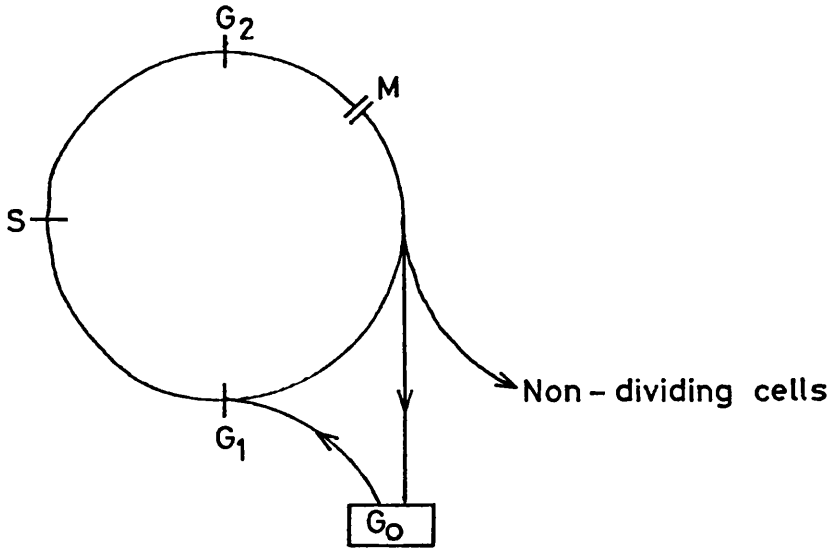
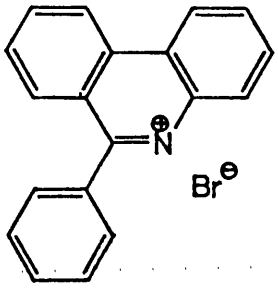


Figure 1. The cell cycle of mammalian cells

Certain cells continuously move around the cycle; others leave temporarily and enter a resting phase, here represented by Go; finally, other cells leave the cycle permanently and die without further division.

The cell cycle is defined as the interval between the mid-point of mitosis of a cell and the midpoint of the subsequent mitosis of one or both daughter cells. At the completion of mitosis (M) the cell spends a variable period of time in a resting phase G_1, during which enzymes required for DNA synthesis are assembled. This accumulation implies that the cell is committed to divide at some future time. An unknown signal stimulates the cell into RNA synthesis and protein synthesis, which is rapidly followed by DNA synthesis (S). Thus the G_1/S interface is not well defined. Drugs which bind to or incorporate into DNA may be lethal to cells in the late G_1 phase since a fully functional DNA template is required for RNA transcription. Such materials include ellipticine (1a, R=H), ethidium bromide (2) and actinomycin D(3)[6]. Inhibitors of protein synthesis show their activity in the late G_1 phase, or G_1/S, examples being cycloheximide (4), puromycin (5) and alkylating agents such as methylmethanesulphonate (6).

In a normal cell cycle lasting between sixteen and forty hours, the phase of DNA synthesis spans a duration of some six to twelve hours. Some drugs administered during the M or G_1 phases may be retained long enough to act during the S phase[7,8]. Lethal S phase inhibitors are reported to be numerous, and include the intercalating drugs daunomycin (7a, R=CH_3), adriamycin (7b, R=CH_2OH), ethidium bromide and actinomycin D. Agents which are incorporated into DNA or interfere with the biosynthesis of nucleotides, such as 5-fluorouridine (8b, R=R), methotrexate (9) and the tubulin-active agents vincristine (10a, R=CHO) and vinblastine (10b, R=CH_3) also show their activity here.



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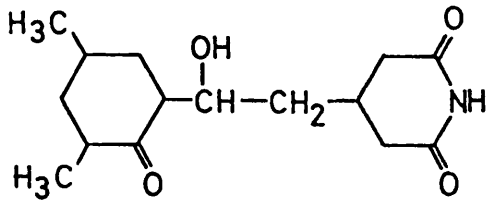

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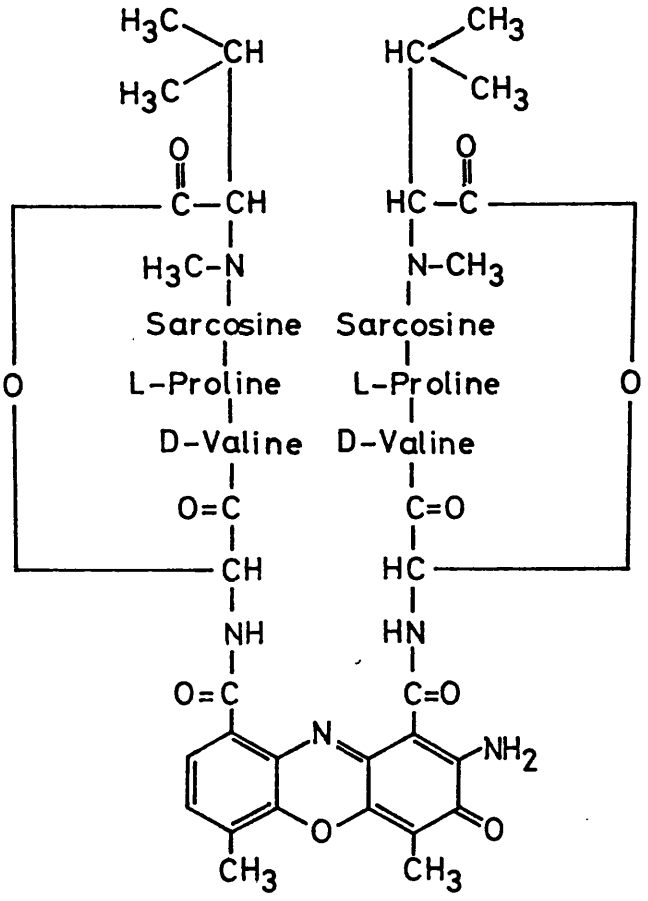

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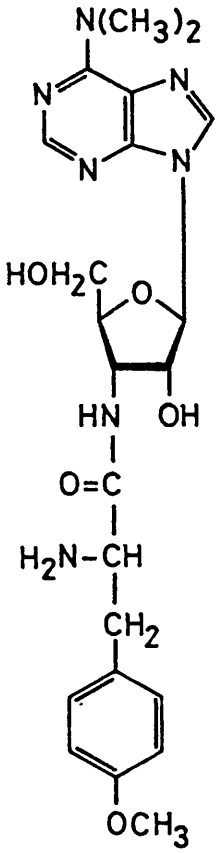

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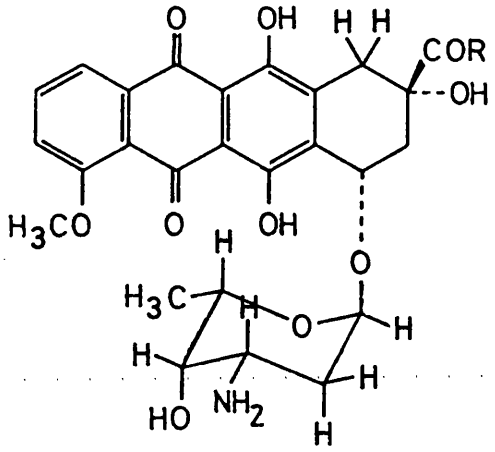
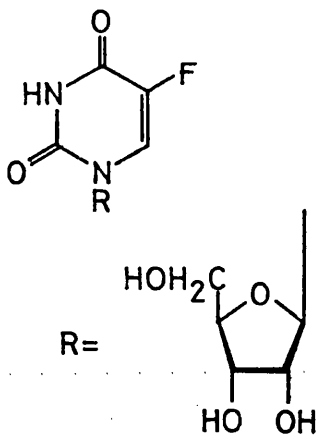
$CH_3SO_3CH_3$

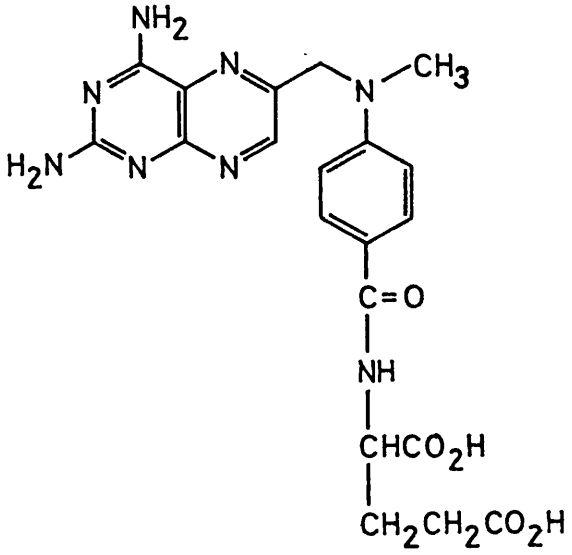
6



7a, R=CH_3
7b, R=CH_2OH



8a, R=H
8b, R=R



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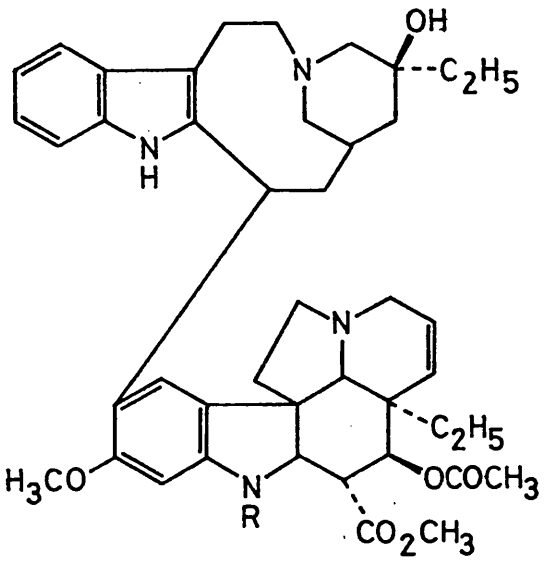
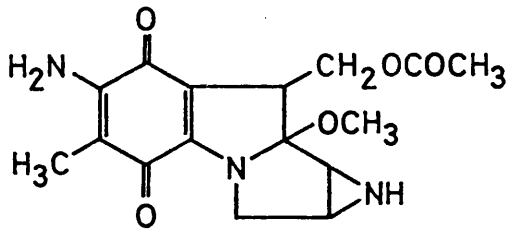


10a, R=CHO
10b, R=CH_3

The G_2 phase usually only extends for a brief period of time, often less than two hours, and immediately precedes mitosis. For the majority of cells the M phase is also comparatively short. It has been speculated[9] that a surveillance mechanism is in operation throughout the G_2 phase which recognises cells bearing altered DNA, and that abnormal cells are retained in a non-viable state until the damaged DNA is repaired, when they are returned to

the cycle. Substances reported[6] to be lethally toxic to cells in the G_2 phase include the fluorinated pyrimidine 5-fluorouracil (8a, R=H) and DNA - reactive drugs like mitomycin C (11) and adriamycin. Each of these compounds is also lethal during the M phase, as well as actinomycin D, ellipticine, vinblastine and various alkylating mustards (12). These phase-specific activities are summarised in Figure 2.



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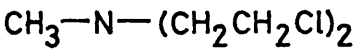

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Many consider that cancer chemotherapeutic agents such as these are not in themselves lethal agents[10], since they are substances which inhibit cell growth rather than exerting a purely cytocidal effect. The pyrimidine analogues such as 5-fluorouracil (8a, R=H) are anti-metabolites which inhibit the incorporation of pyrimidines[11]. Similarly, methotrexate (9) is a folic acid antagonist which blocks a pathway in the intermediary metabolism through competition for the enzyme binding site in folate reductase. This substance normally converts folic acid into an intermediate which is essential for the biosynthesis of the nucleic acid bases later incorporated into DNA and RNA.

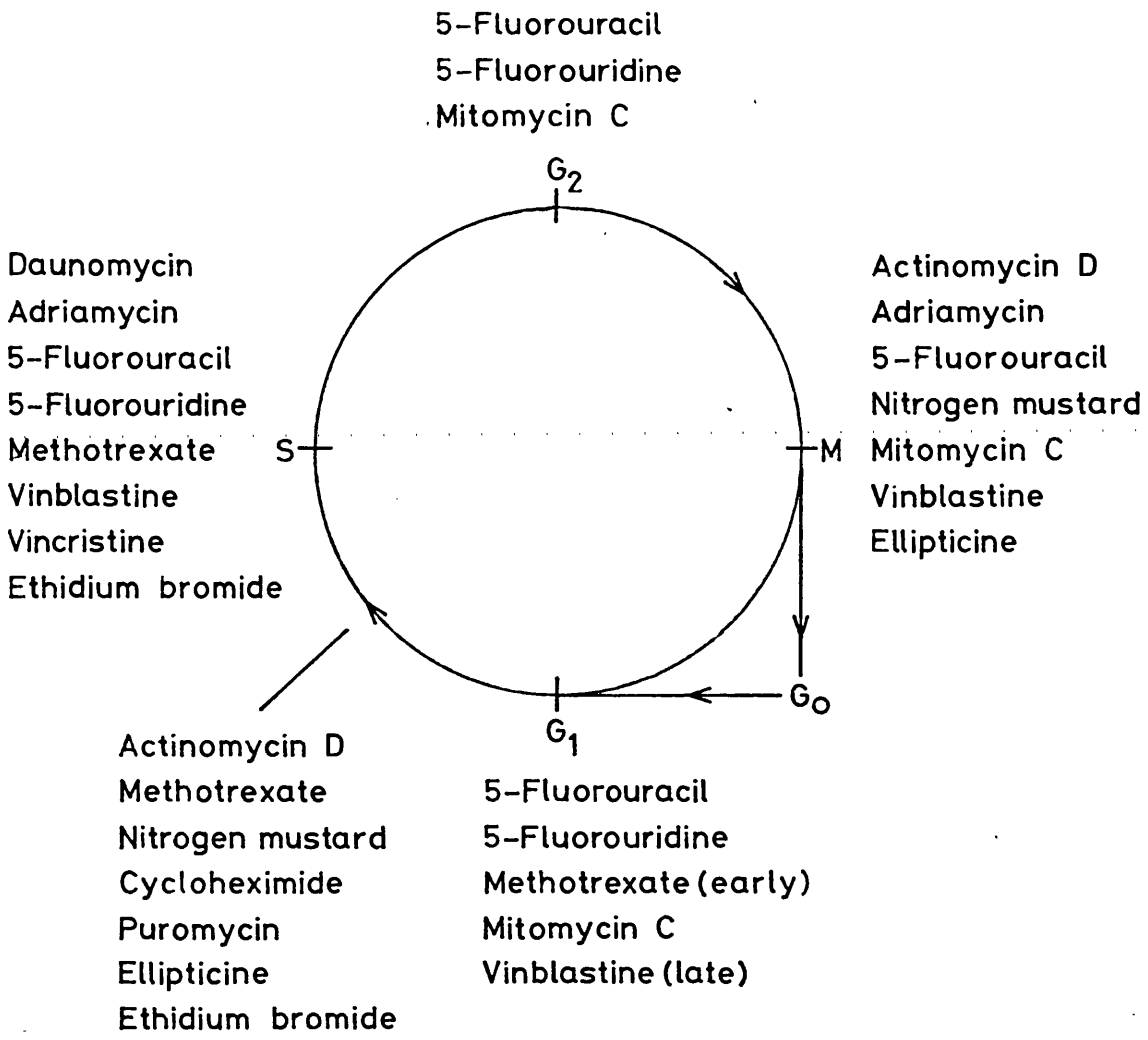


Figure 2. The cell cycle, showing where phase-specific antitumour agents exert their lethal effect[6]

The alkylating agents, of which nitrogen mustard (12), or mechlorethamine, is probably the most illustrative example, are among the oldest cancer chemotherapeutic agents and perhaps their mechanism of action is more readily understood. Whilst it is true that enzymes may be destroyed by alkylation, this is not thought to be the primary action of the administered drug, since one of the earliest observations was the similarity between the effects of X-rays and nitrogen mustards, both of which lead to disruption of the nuclear material in the cell. Thus it becomes clear that these substances inhibit cell growth and proliferation because they alkylate the nucleic acids, and in particular they have been

shown to react with guanine at position 7[12]. The presence of the alkyl group on the nucleo-protein then completely destroys its function.

The mode of action of other drugs is less well-defined. Whilst tests have shown[7,8] that ellipticine is active throughout all phases of the cell cycle, and particularly so during the M and G_1 phases,the mechanism by which this is achieved is not entirely clear. Like many other substances having a planar structure, ellipticine is believed to interact with DNA by intercalation, thereby distorting its structure and diminishing its function. Waring[13] describes three such modes of action for certain anti-microbial drugs, some of which also find use as antitumour agents for the same reason.

The fact that ellipticine is an intercalating drug is beyond question since this type of interaction manifests itself by inducing measurable changes in a number of parameters. In particular, a large increase in viscosity is observed, and X-ray diffraction shows that although the close-range stacking of the base-pairs in DNA is unimpaired, the longer range ordering or supercoiling of the molecule is lost. This is reflected by changes in the so-called unwinding angle[14].

The size, planarity and the arc-shaped form of ellipticine molecules are believed to be particularly favourable characteristics for DNA intercalation. The specific electronic configuration is also important because this determines the magnitude of the energy of interaction. In some way the ideal drug must be a mould of the cavity formed in DNA when two adjacent base-pairs become disengaged due to the dynamic structure of DNA[15]. If the electronic characteristics of the drug material are favourable, it

may become bound to this site through hydrogen bonding with appropriately positioned base-pairs of the DNA spiral or it may be secured by covalent bonding brought about by secondary chemical reaction *in situ*. Thus 9-methoxyellipticine is believed to undergo *in vivo* conversion to the form illustrated in Figure 3.

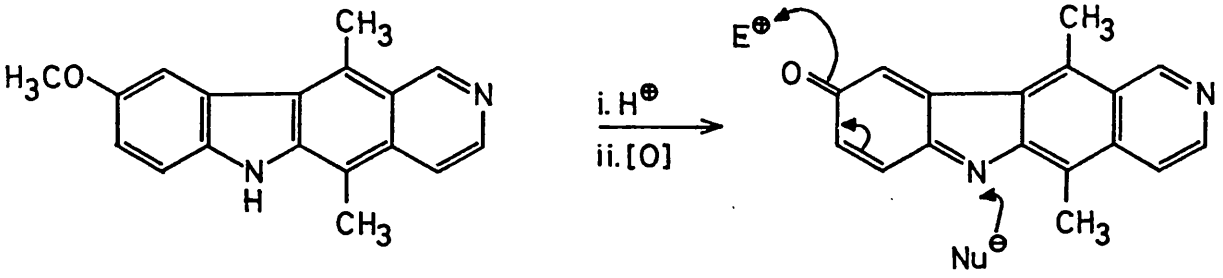


Figure 3. The structure of 9-methoxyellipticine in the living cell

Le-Pecq and his colleagues[16] have quantified the DNA binding affinity of a number of ellipticine derivatives in an effort to rationalise the structure/activity relationships. As a result of this work they synthesised 9-hydroxyellipticine and demonstrated that it has a high activity against the murine L1210 leukaemia and a low toxicity at therapeutic dose levels. Their results are summarised in Table 1.

In solution at physiological pH 7.4, ellipticine derivatives exist in both neutral and protonated forms. Under these conditions the apparent DNA binding constant, which is the measured value, is dependent on the respective fractions of the protonated and neutral forms. In other words the apparent binding constant, K_{AP}, is proportional to the pK_a of the molecule. In order to study other factors affecting the DNA affinity, the binding affinity of the cationic form of the drug, K_{E^+}, must be considered on its own,

since this is independent of pK_a. In general the value of K_{E^+} is about thirty times greater than the DNA affinity of the neutral form. Then if $pK_a >> pH$, the apparent binding affinity approximates to the binding affinity of the protonated drug materials.

Table 1. DNA-binding affinity and pharmacological activity for some ellipticine derivatives[17]

Ellipticine substituent	pK	K_{AP} (pH 7.4)	log K_{E^+}	Unwinding angle	Activity[d.]
6-Isopentyl	4.7	$<10^4$ [a.]	6.3	8.8[b.]	0
6-Isopentyl-9-methoxy	4.5	$<10^4$ [a.]	6.7	-	-
5,11-didemethyl	6.35	1.0×10^4	5.08	-	0
11-Demethyl	6.3	2.4×10^4	5.52	-	0
9-Methoxy	6.8	1.0×10^5	5.7	6.8	90
Unsubstituted	9.1	1.5×10^5	5.7	9	94
9-Bromo	6.1	4.0×10^5	6.92	0[c.]	0
6-Methyl	6.1	4.0×10^5	6.92	10.2	92
9-Amino	9.8	1.2×10^6	6.08	4	0
6-Methyl-9-methoxy	6.45	2.0×10^6	7.3	5	50
9-Hydroxy	9.8	2.0×10^6	6.15	12	99.96

a. These compounds are insoluble at pH 7.4. Log K_{E^+} is deduced from measurements of K_{AP} made at pH 5.0.
b. Measurement made at pH 5.0.
c. 9-Bromoellipticine does not intercalate.
d. The pharmacological activity is expressed as the percentage of L 1210 cells killed by one third of the LD_{50}.

From measurements to determine the ability of ellipticine derivatives to compete with the binding of ethidium bromide in DNA, the French workers derived the following expression for the apparent DNA binding constant:

$$\log K_{AP} = \log K_{E^+} - \log \left(1 + \frac{K_H^{-1}}{[H^+]}\right) + \log \left(1 + \frac{1}{a} \cdot \frac{K_H^{-1}}{[H^+]}\right)$$

Here K_H-1 is the dissociation constant of the equilibrium between protonated and neutral forms of the drug, and α is the ratio of the DNA binding constants of the protonated and neutral forms.

Regrettably the compounds tested by the French team are illustrative of the difficulty with which ellipticine derivatives are synthesised. In order to establish a relation between the DNA reactivity of ellipticine derivatives and their anti-cancer properties it is necessary to undertake a thorough study of a large number of compounds, both active and inactive. In particular, it is important to consider what type of metabolic modifications, if any, may be taking place. The significance of *in vivo* modification is illustrated in the table by 9-aminoellipticine, which is inactive against L1210 cells although it has a higher affinity for DNA than ellipticine itself. Clearly DNA binding affinity alone is not a sufficient criterion for anti-tumoural activity, but it is probably a necessary condition.

In the succeeding pages synthetic efforts towards ellipticine derivatives are briefly reviewed. A more comprehensive treatment of the synthesis of 6*H*-pyrido [4,3-*b*] carbazoles in general may be obtained from a study of the review by Sainsbury[19].

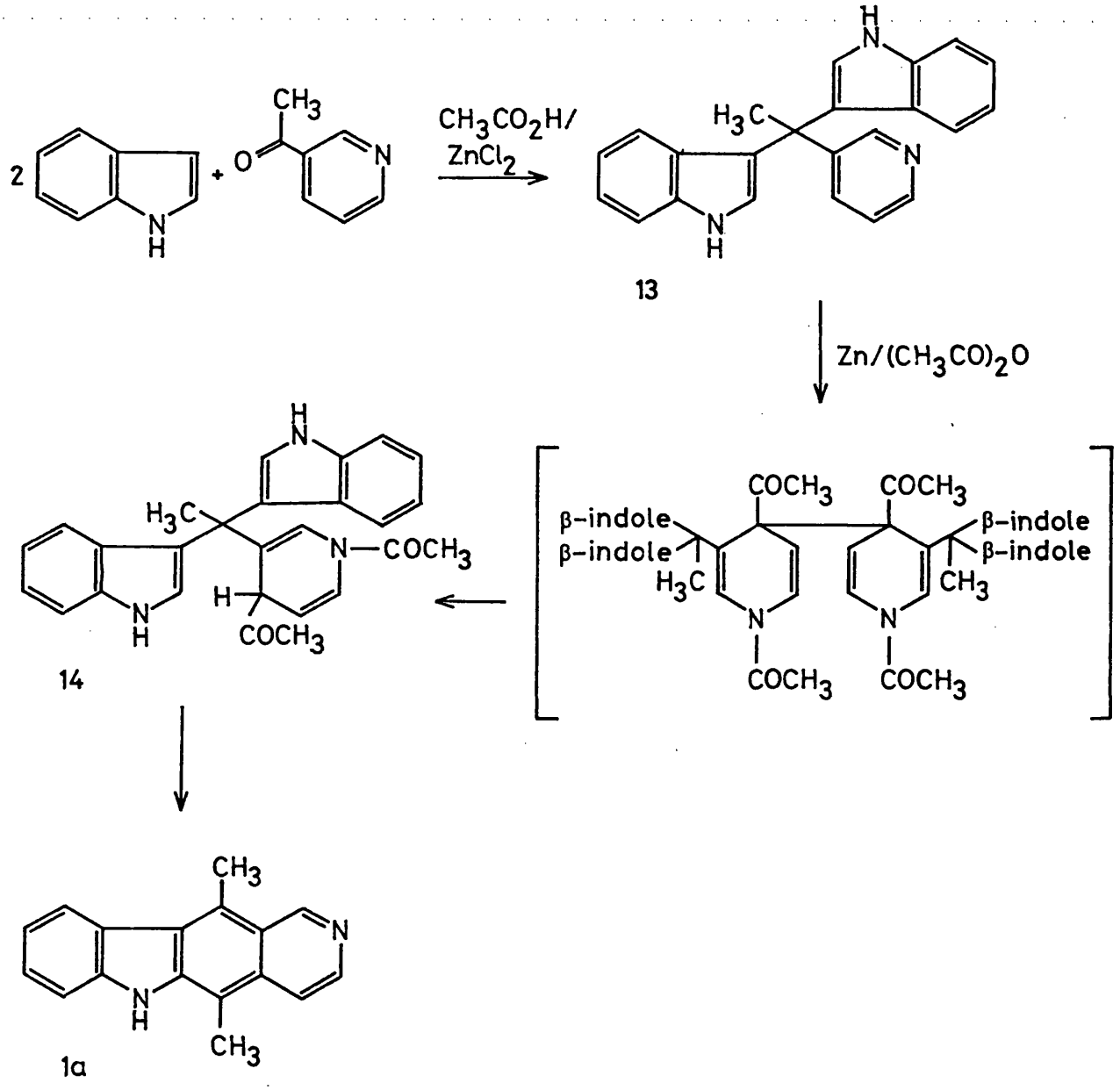
Synthesis of ellipticine derivatives:

The first synthesis of ellipticine was carried out by Woodward, Iacobucci and Hochstein[20], immediately following its isolation by Goodwin et al[1]. This method served to determine the structure of the alkaloid, but whilst it is a simple and direct route (see Scheme 1), it is of little synthetic value since the overall yield of the product is poor. Condensation of indole and 3-acetylpyridine in acetic acid in the presence of zinc chloride yields 1,1-bis-(3-indolyl) - 1 - (3-pyridyl) ethane (13). This 2:1 product is a bulky molecule which is clearly a poor substrate for the ensuing reductive acetylation procedure which is carried out with zinc in refluxing acetic anhydride. This process leads to a sterically hindered dimeric species which then undergoes disproportionation to give unchanged starting material and the dihydropyridine (14). The efficiency of this sequence is further diminished by the final oxidative ring closure step which requires severe pyrolytic conditions to remove the additional indolyl unit. Ellipticine is thus obtained in only 2% yield.

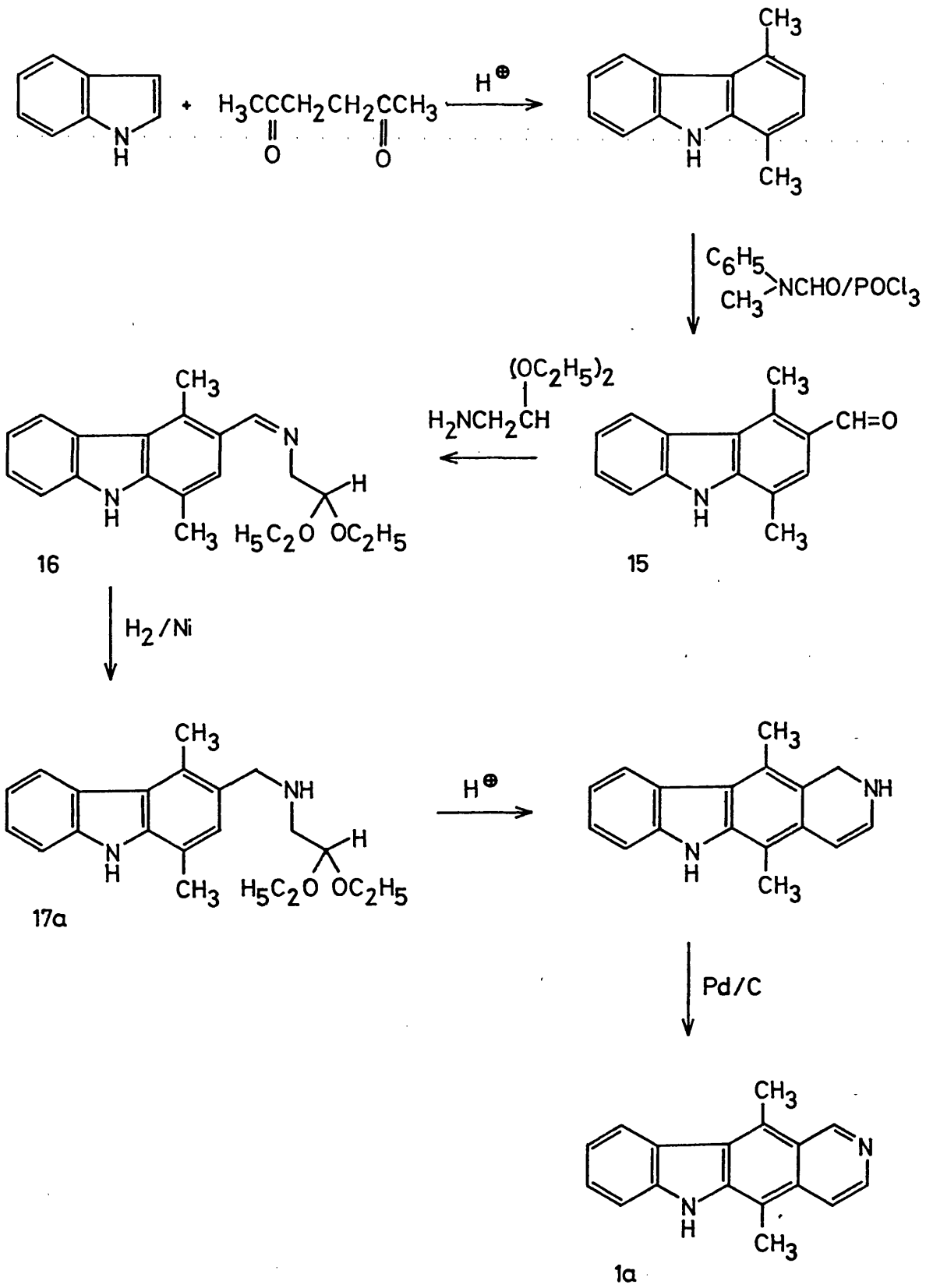
It was some three years later that a second synthesis of ellipticine was published by Cranwell and Saxton[21]. This method (see Scheme 2) was welcomed as a more versatile route employing less severe conditions and certainly more sterically favourable substrates. Initially, 1,4-dimethylcarbazole is prepared from indole and 2,5-hexanedione. This undergoes formylation in a Vilsmeier reaction to give 3-formyl-1,4-dimethylcarbazole (15) which is condensed with 2,2-diethoxyethylamine to yield the Schiffs base (16). Attempts to convert this directly to ellipticine using the Pomeranz-Fritsch technique proved unsuccessful. However, the corresponding saturated acetal (17a) is readily cyclised with dry hydrogen chloride to 1,2-dihydroellipticine

and the final dehydrogenation step is carried out by heating with palladium on charcoal to give ellipticine in about 1.5% yield relative to indole.

Scheme 1.



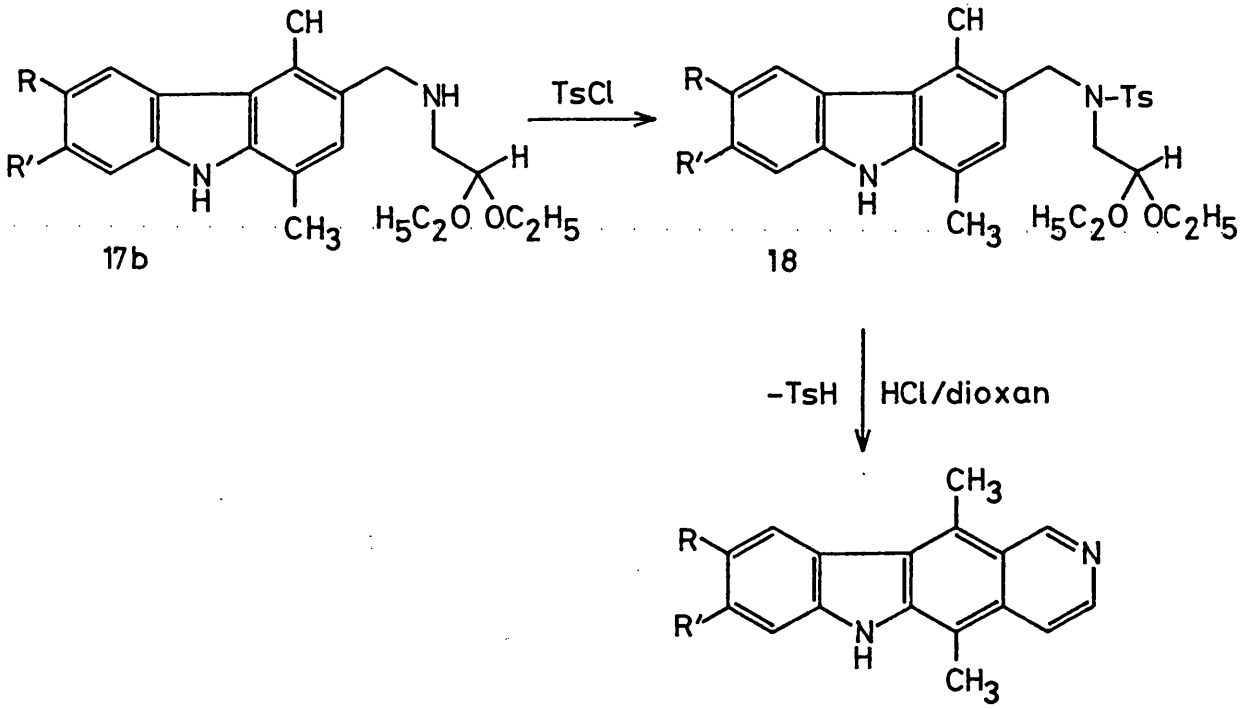
Scheme 2.


H⊕
C6H5
CH3 NCHO/POCl3
(OC2H5)2
H2NCH2CH
CH=O
16
15
H2/Ni
17a
H⊕
Pd/C
1a

The potential of this method was first explored by Dalton's team[22] who realised that the final two steps of this sequence could be avoided by using orthophosphoric acid to effect direct cyclisation of the azomethine (17a) to ellipticine. By means of this modification Dalton et al. were able to prepare ellipticine in improved yields, as well as several other derivatives variously substituted about the indole skeleton. Unfortunately it is found that electron - withdrawing groups in ring A lead to a decrease in the amount of ring closed product. Although electron - donating groups increase the yield of ring closed product, this also leads to the formation of a mixture of formylated compounds and carbazoles may be isolated which are formylated in the A ring itself. Nevertheless, as an illustration of the versatility of this method, Elmes and Swan[23] were able to prepare 6-oxaellipticine and related heterocycles from 1,4-dimethyldibenzofuran and corresponding starting materials.

A second variation on Cranwell and Saxton's synthesis was devised by Guthrie and his co-workers[24], employing a modification of the Pomeranz - Fritsch isoquinoline synthesis first reported by Birch, Jackson and Shannon[25]. Here the dihydroazomethine (17b), prepared in the usual manner, gives the corresponding N-tosyl derivative (18) when reacted with p-toluenesulphonyl chloride (see Scheme 3). Treatment of this compound with hydrochloric acid in dioxan results in cyclisation to ellipticine with elimination of p-toluenesulphinic acid.

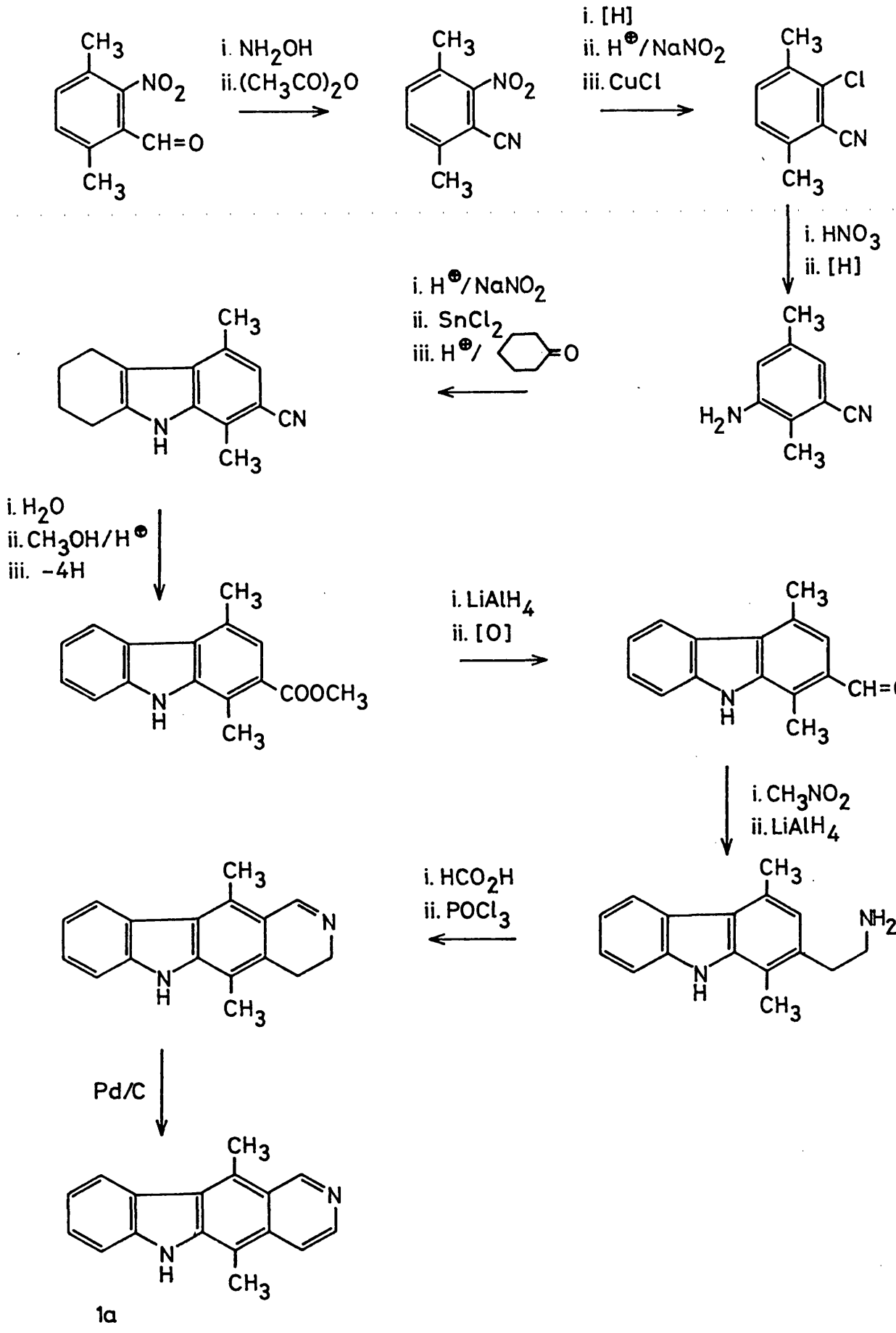
Scheme 3



Amongst the earliest reported syntheses of ellipticine is that of Govindachari, Rajappa and Sudarsanam[26]. This multiple stage route (see Scheme 4) is a wonderful example of classical organic chemistry, but is of little use as a general preparative method for variously substituted derivatives because of the large number of steps involved.

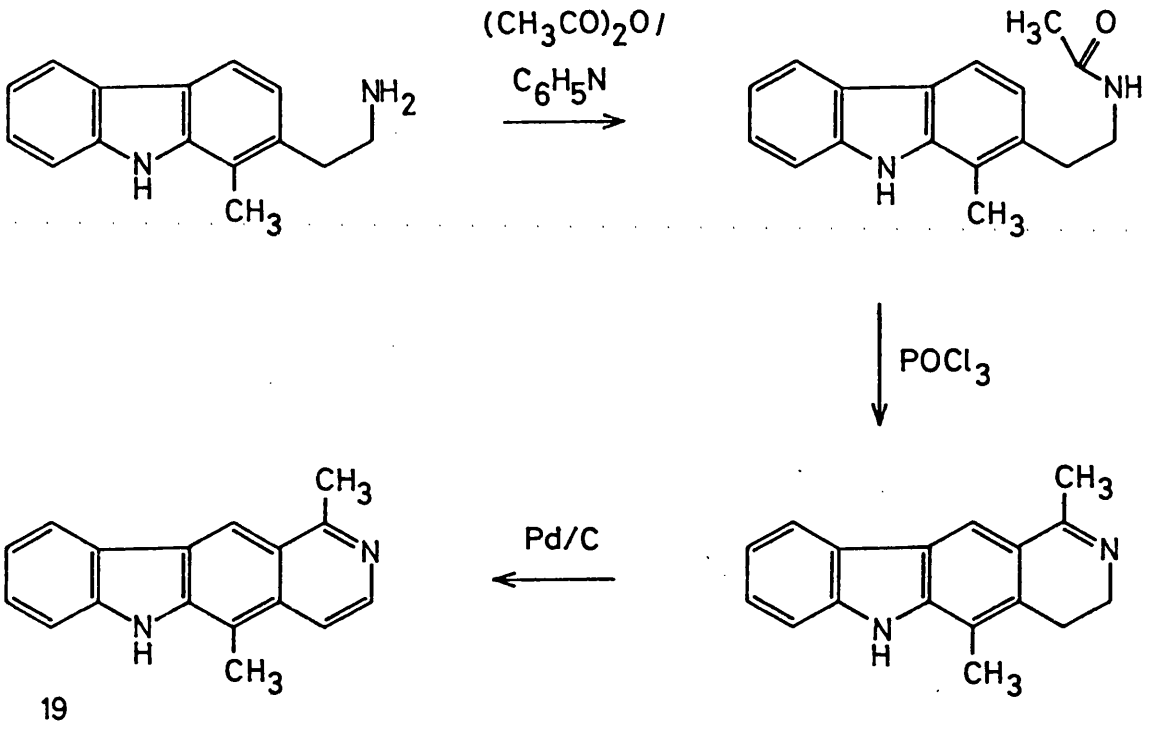
The latter stages of this scheme are similar in outline to Mosher's synthesis[27] of the isomeric alkaloid olivacine (19) (see Scheme 5), which is itself a slight modification of the method of Schmutz and Wittwer[28]. A Swiss patent filed by Wander[29] describes this route in much more general terms, allowing for various substituents to be attached to the pyridocarbazole skeleton.

Scheme 4


i. NH2OH
ii.(CH3CO)2O
i. [H]
ii. H⊕/NaNO2
iii. CuCl
i. HNO3
ii. [H]
i. H⊕/NaNO2
ii. SnCl2
iii. H⊕/
i. H2O
ii. CH3OH/H⊕
iii. -4H
i. LiAlH4
ii. [O]
i. CH3NO2
ii. LiAlH4
i. HCO2H
ii. POCl3
Pd/C

1a

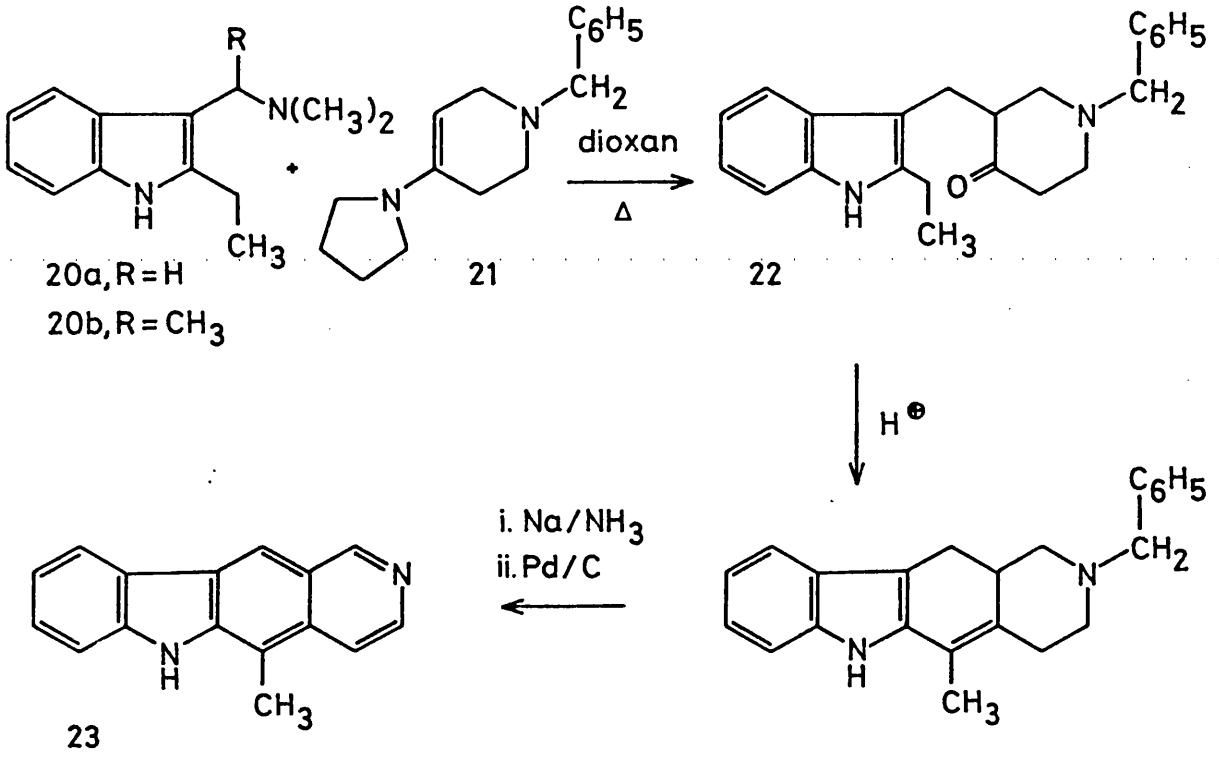
Scheme 5



Early in 1973 a team of French workers led by Le Goffic[30] described two new syntheses of the 6H-pyrido [4,3-b] carbazole system. In the first of these methods (see Scheme 6) 2-ethylgramine (20a, R=H) is combined with the enamine (21) by refluxing in anhydrous dioxan to give the (2-ethylskatyl) piperidone (22). Cyclisation of this species is brought about by refluxing in acetic acid, and the fully aromatic 11-demethylellipticine (23) is obtained after debenzylation and dehydrogenation.

In trying to synthesise ellipticine itself by this method, a problem arose in the preparation of the desired starting material (20b, R=CH_3). Attempts to obtain this from the reaction of 2-ethylindole with acetaldehyde and dimethylamine were unsuccessful, resulting in products of the 1,1-bis-(3-indolyl) ethane type from a modified Mannich reaction. To circumvent this

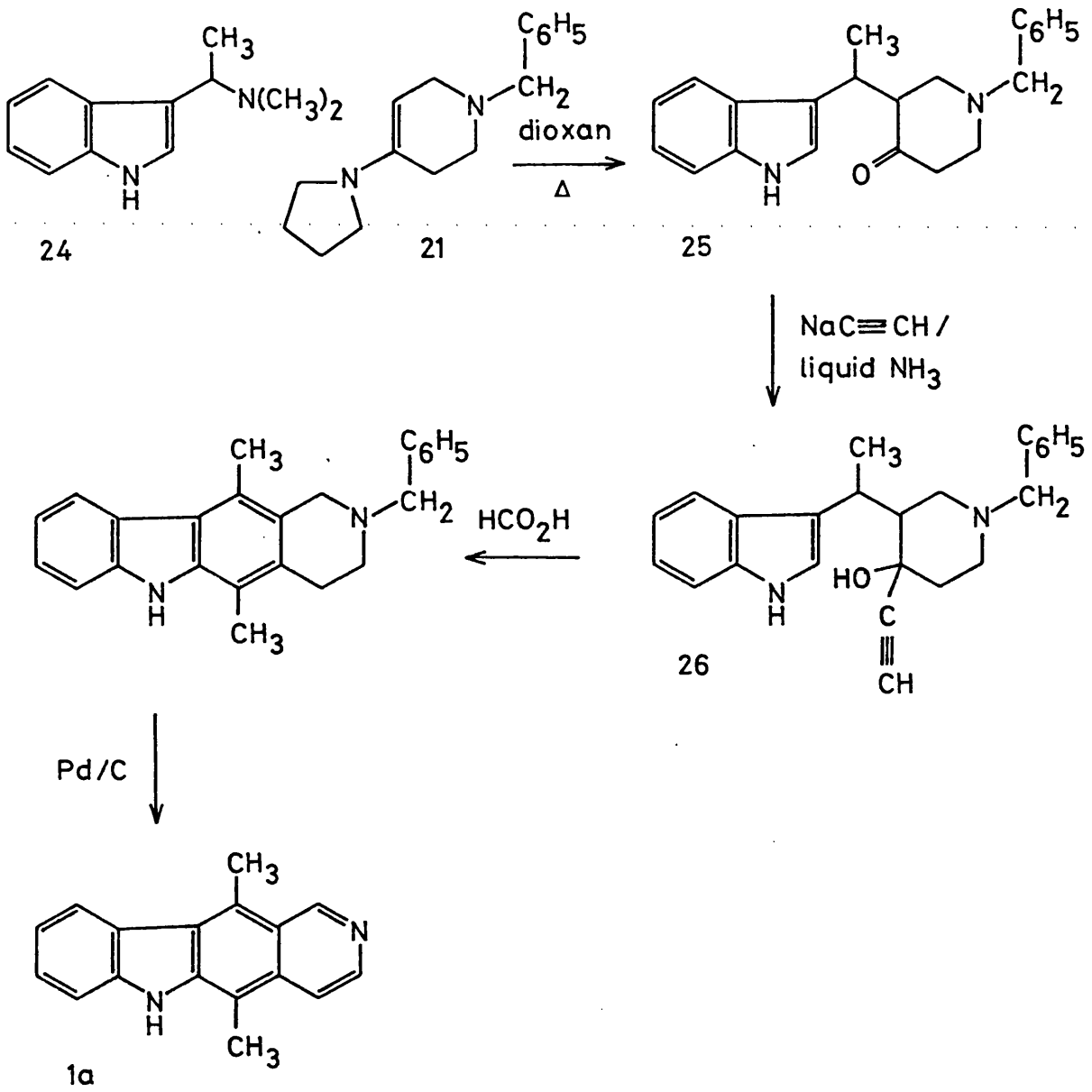
Scheme 6



problem an alternative route was devised (see Scheme 7) in which the starting material (24), a methyl derivative of gramine, is prepared through the condensation of dimethylacetamide and indole under Vilsmeier-Haack conditions, with subsequent reduction of the resulting imminium salt using sodium borohydride. This material is reacted with the enamine (21) to give the piperidone (25) and this in turn is combined with sodium acetylenide in liquid ammonia to give the acetylenic alcohol (26). On treatment with formic acid, 2-benzyl -1,2,3,4 - tetrahydroellipticine is formed and this may be debenzylated and dehydrogenated to give the parent alkaloid in 24% yield from indole.

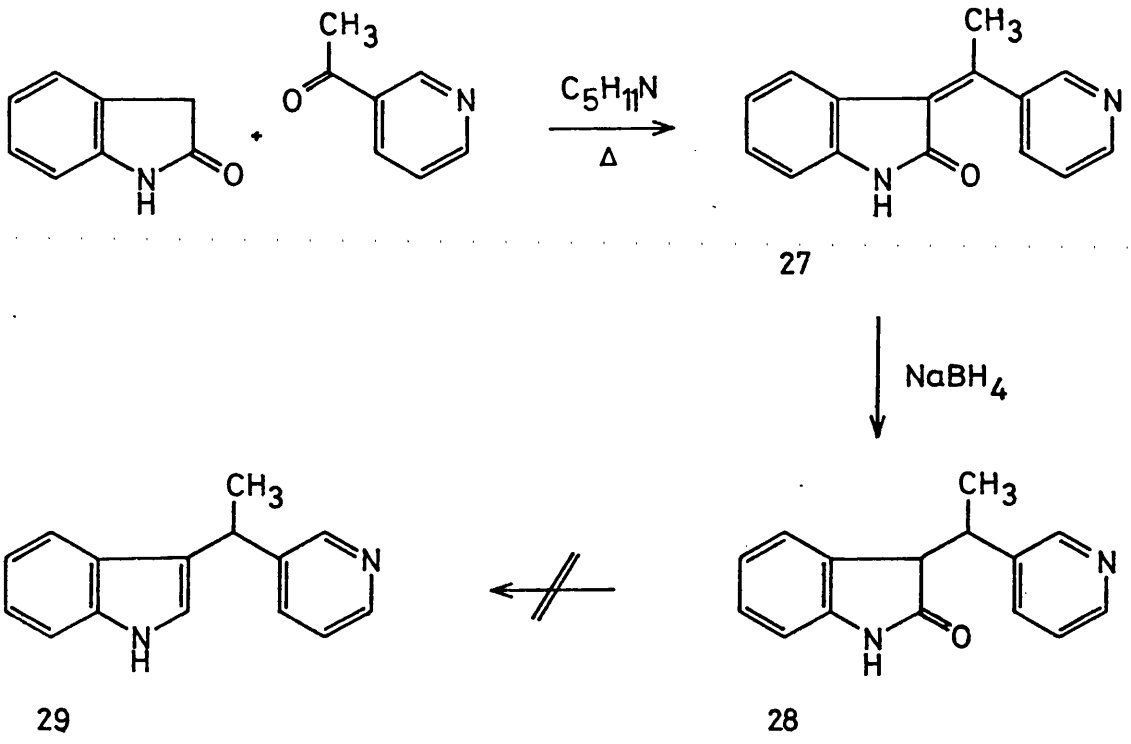
In each of these schemes the major drawback is the final dehydrogenation step which may result in defunctionalisation when labile substituents are present.

Scheme 7



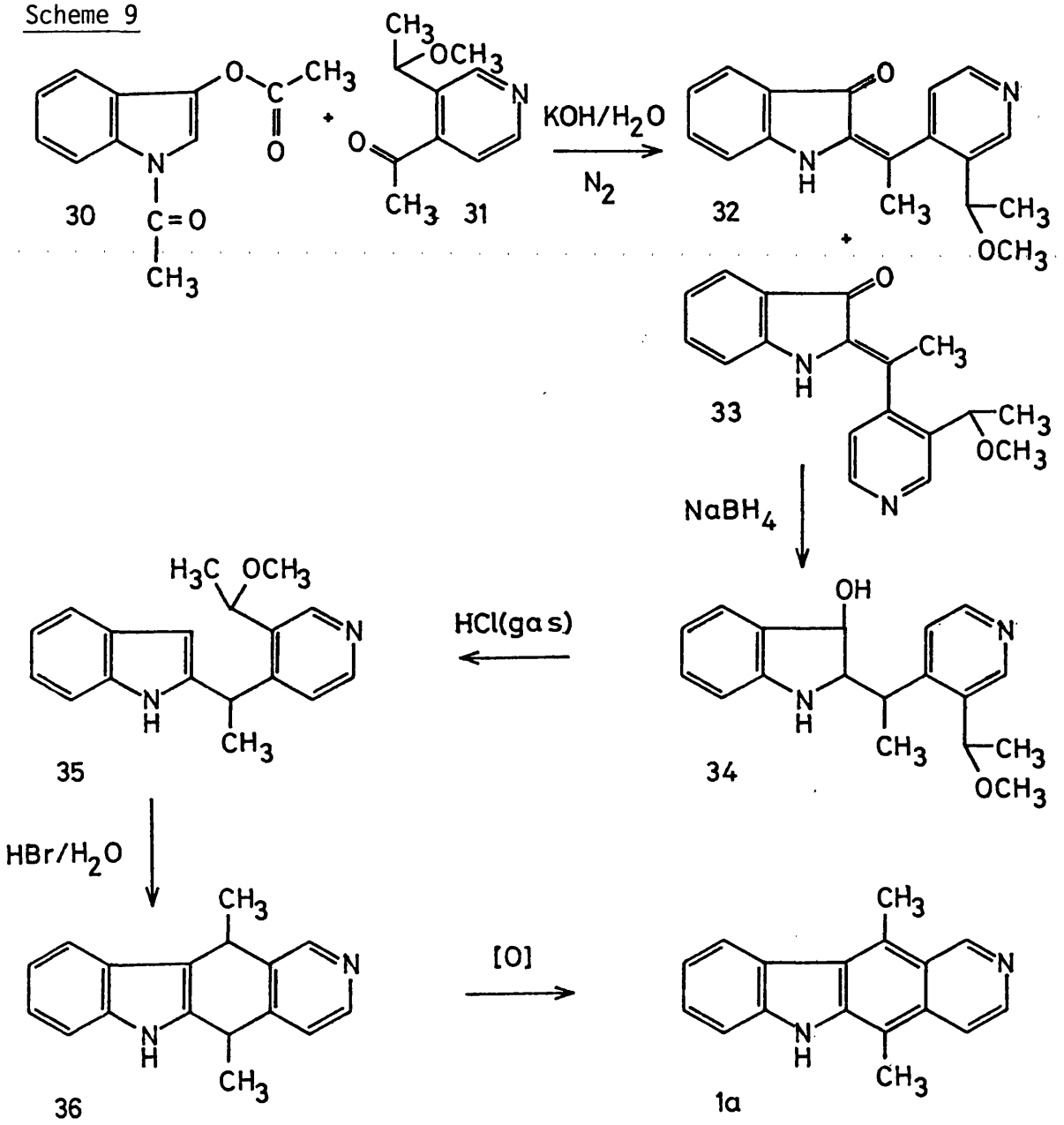
In an attempt to overcome the problem of formation of bis-indolyl compounds, Kilminster and Sainsbury[31] reacted 3-acetyl-pyridine with oxindole to give a 1:1 adduct (27) (see Scheme 8). This oxo-indoline is actually obtained as a mixture of E - and Z - isomers, and whilst this is readily converted to the dihydro derivative (28) with sodium borohydride in aqueous methanol, further reduction to the desired 3-[1-(3-pyridyl)ethyl] indole (29) cannot be induced in acceptable yield owing to the strong amide character.

Scheme 8



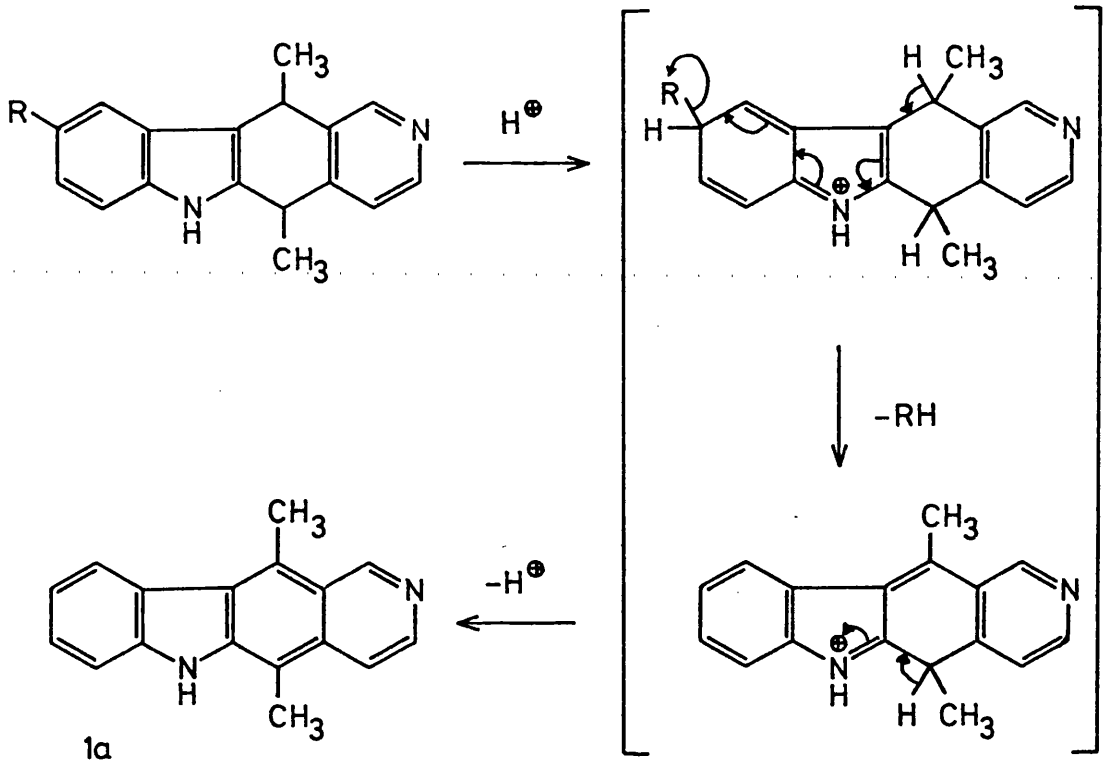
As an alternative procedure, the use of 1-acetylindol -3 - ol acetate (30) and 4-acetyl - 3 - (1-methoxyethyl) pyridine (31) as starting materials was proposed [32,33](see Scheme 9). These are condensed under a nitrogen atmosphere in the presence of methanolic potassium hydroxide to give 2-{1-[3-(1-methoxyethyl)-4-pyridyl] ethylidene}indolin - 3 - one as its E- and Z- isomers (32 and 33) which are reduced with sodium borohydride to the alcohol (34). This is treated with hydrogen chloride in chloroform to give 2-{1-[3-(1-methoxyethyl) - 4 -pyridyl] ethyl} indole (35) by elimination of water. Cyclisation to 5,11-dihydroellipticine (36) is brought about by refluxing in 40% aqueous hydrogen bromide and oxidation to ellipticine is spontaneous when the product is eluted through a column of silica gel using chloroform as eluent.

Scheme 9



In this synthesis, aromatisation of the dihydroellipticine may be a troublesome step with respect to certain 9-substituted ellipticines. When the substituent is a good leaving group, the required product is often contaminated with a considerable amount of unsubstituted ellipticine. The oxidative elimination is believed to proceed by means of the mechanism outlined over. (Scheme 10).

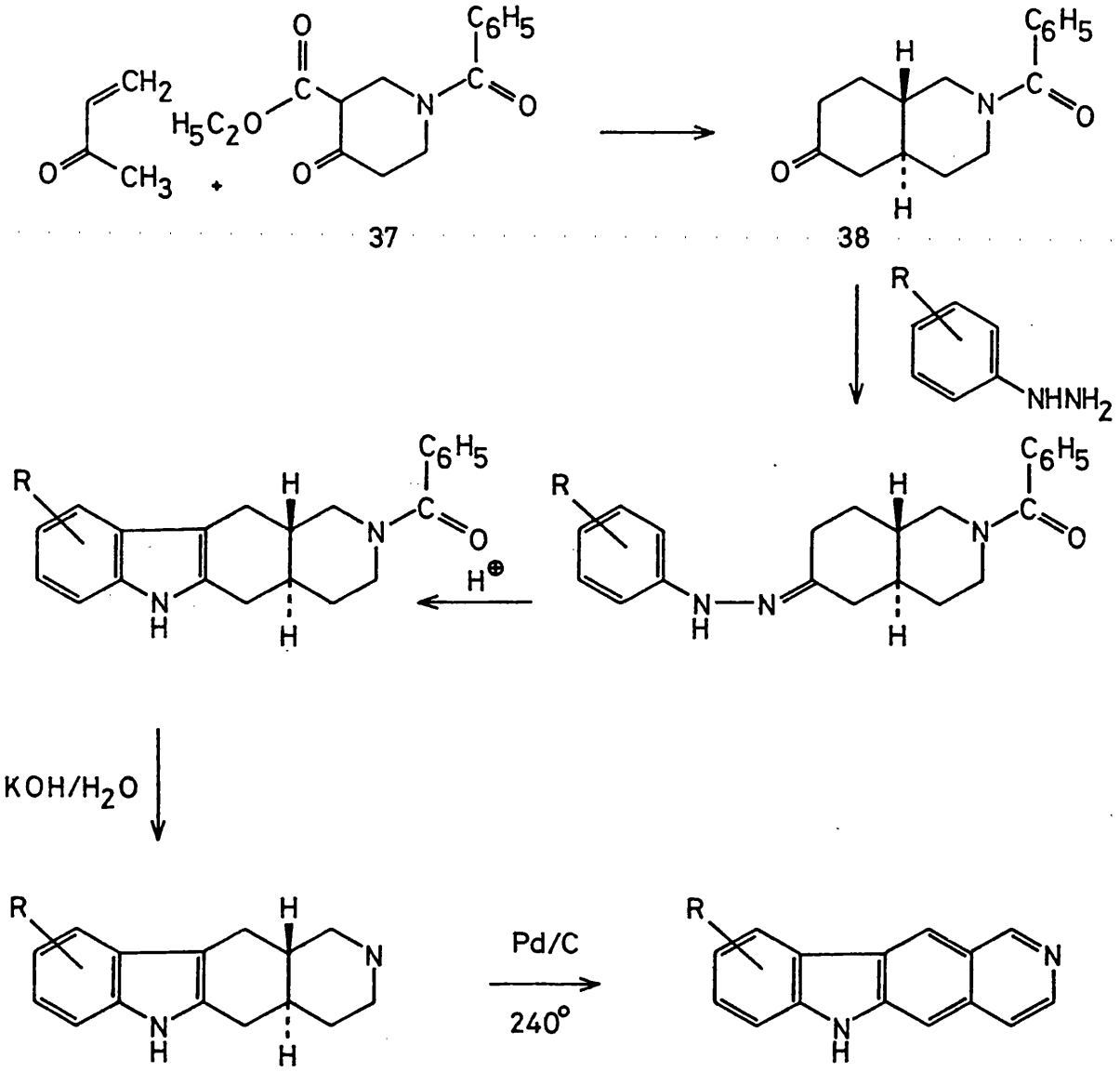
Scheme 10



1a

A number of 6H-pyrido [4,3-b] carbazole derivatives bearing substituents in the A ring were prepared by Rastogi et al. using a method published in 1972[34] (See Scheme 11). Here the key starting material is 2-benzoyl-1,3,4,4a,5,7,8,8a -octahydro-6 (2H)-isoquinolone (38) which is prepared from 1-benzoyl-3-ethoxycarbonyl-4-piperidone (37) and methyl vinyl ketone with subsequent hydrogenation of the product. The octahydroisoquinolone (38) is converted to its phenylhydrazone and this undergoes a Fischer indolisation step, and following debenzoylation the product is dehydrogenated to the fully aromatic derivative.

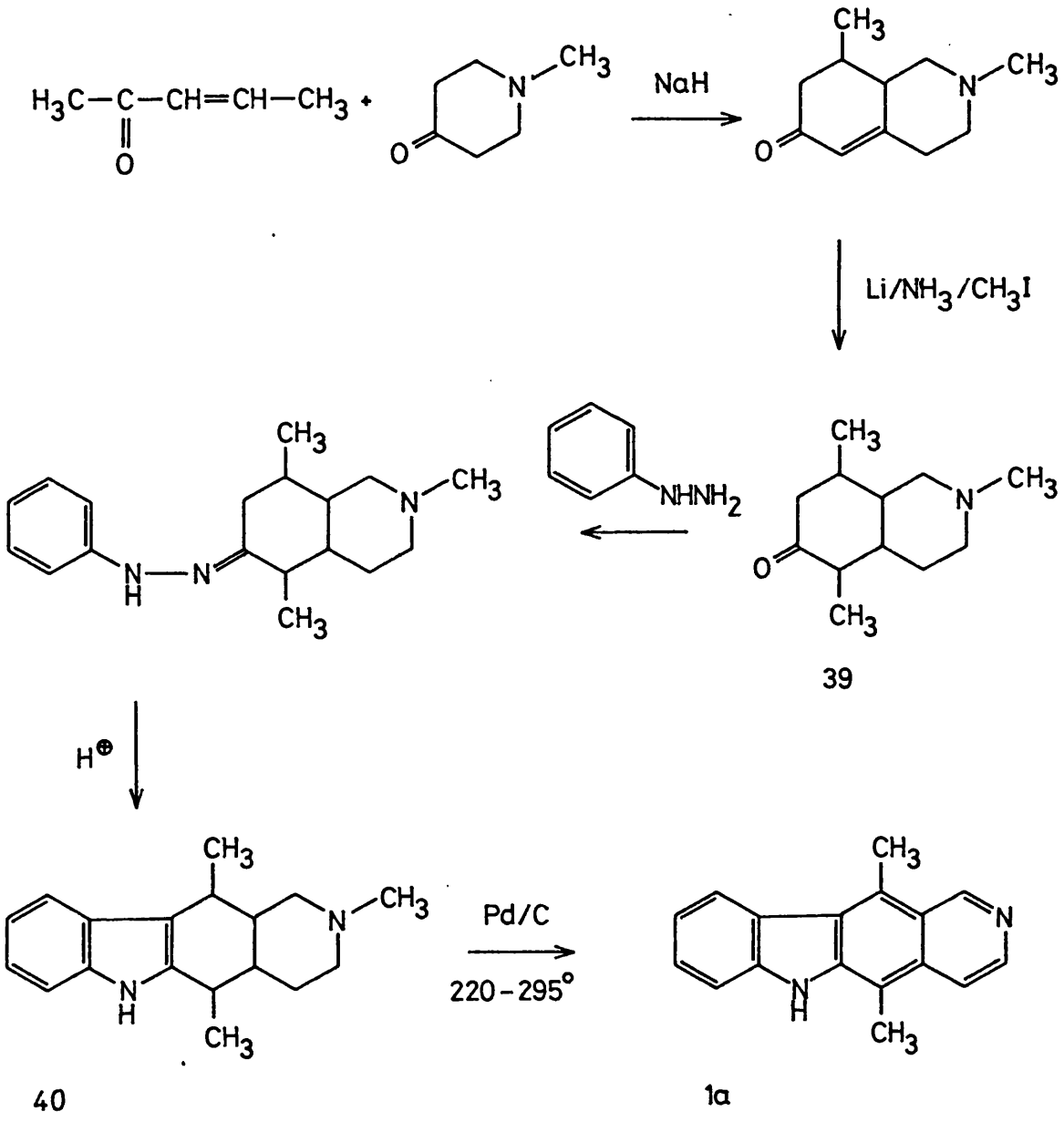
Scheme 11



This method is similar to that used by Stillwell[35] to synthesise 5-demethylellipticine and ellipticine itself. In this route (see Scheme 12) the appropriate decahydroisoquinol-6-one (39) is prepared by a reductive Stork alkylation ~~[of 1-methyl-4-piperidone]~~ using iodomethane in the presence of lithium in liquid ammonia. Once again a Fischer indolisation reaction is carried out on the phenylhydrazone derivative to give 2-methyl-1,2,3,4,5,5a,11,11a - octahydroellipticine (40) which is dehydrogenated to ellipticine by

heating with palladium on charcoal. The quoted yield here is only 0.3%, and whilst most of the other steps in the synthesis work well, the final dehydrogenation step certainly does not. The same remark is almost certainly true with respect to the Indian group's work outlined above, but their publication does not mention the yields which these chemists obtained.

Scheme 12

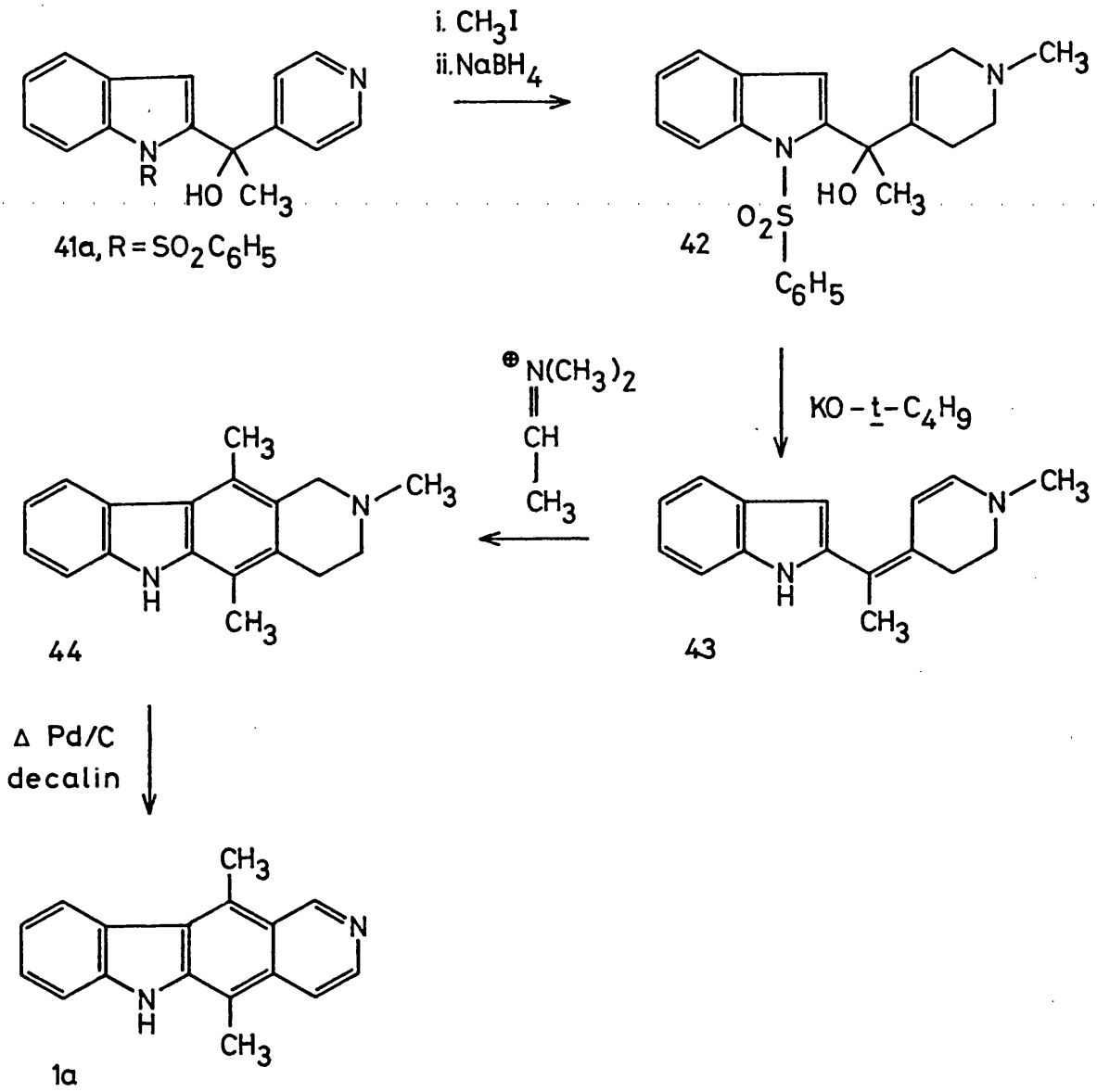


Of particular interest because of their assumed similarity to the biosynthesis of ellipticine are the methods devised by Potier et al.[36,37] wherein the penultimate ring closure step is believed to proceed via an intermediate immonium ion such as (46) or (47). In the first method (see Scheme 13) the starting material (41a, $R=SO_2-C_6H_5$) is prepared by the condensation of 4-acetylpyridine with 1-phenylsulphonyl-2-lithioindole. Iodomethylation of the starting material gives the corresponding pyridinium salt which is reduced to the tetrahydropyridine (42) with sodium borohydride. This is treated with potassium tertiary-butoxide in dimethyl sulphoxide to give the dienamine (43) as its E- and Z- isomers. This product is heated in acetic acid with the Mannich reagent formed by the condensation of acetaldehyde and dimethylamine to give 2-methyl-1,2,3,4-tetrahydroellipticine (44). Ellipticine is obtained by treatment with palladium on charcoal in refluxing decalin.

Alternatively the starting material is hydrolysed to the indole (41b,R=H), and the methiodide salt of this compound is reduced with sodium borohydride in the presence of a large excess of potassium cyanide to give the cyanotetrahydropyridine (45). The product is then reacted with the same Mannich reagent to give the immonium salt (48) via (46) or (47). Without isolation, this product is reduced with sodium borohydride to 2-methyl-1,2, 3,4-tetrahydroellipticine (44)in 24% yield from the cyanide (45). (See Scheme 14).

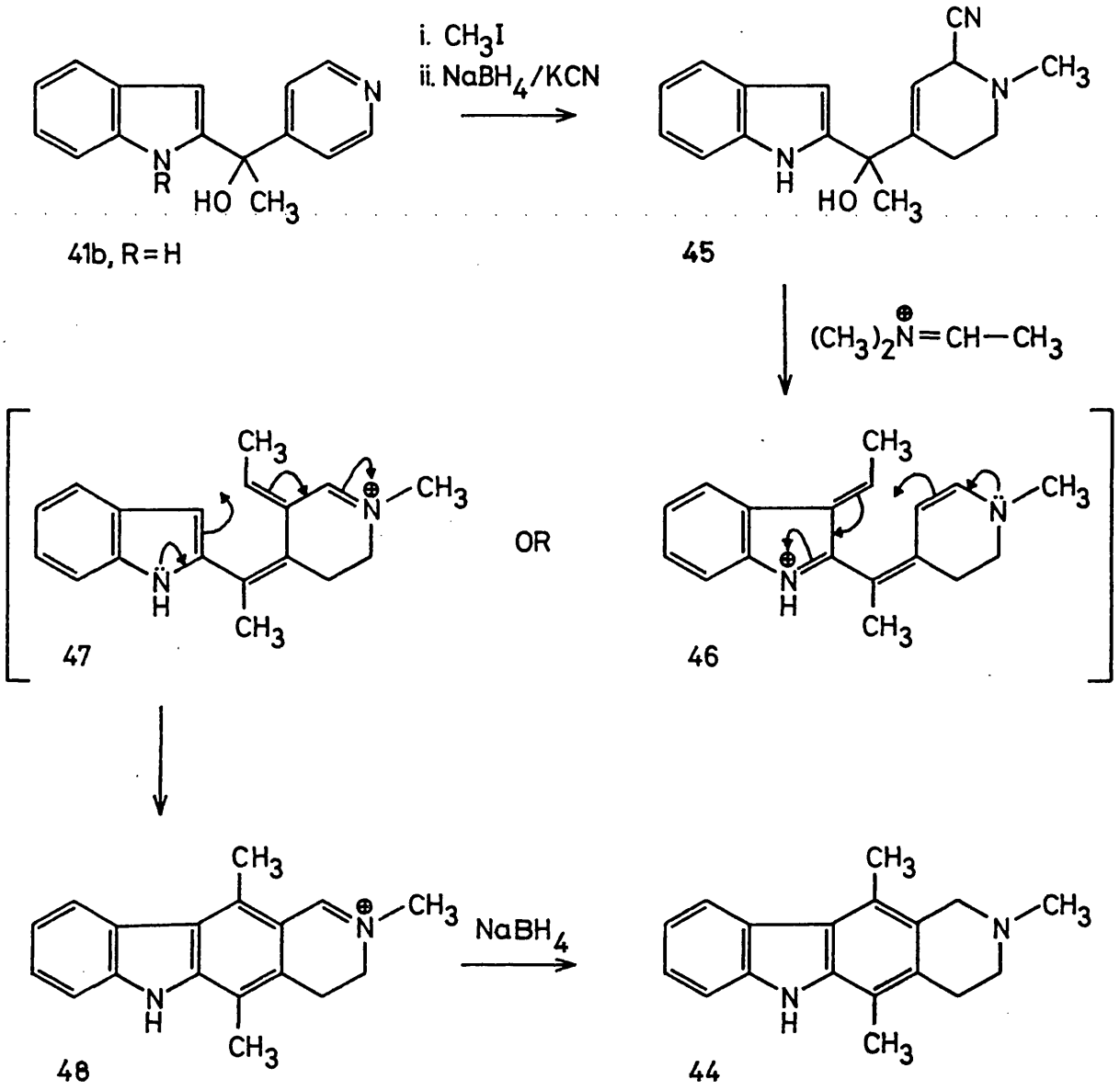
In the third approach[37], the phenyhydrazone derivative (50) of the ketone (49) is subjected to a Fischer indolisation reaction which leads to the indole (51) (see Scheme 15). The $N_{(b)}$-oxide of this compound is treated with trifluoracetic anhydride to effect

Scheme 13



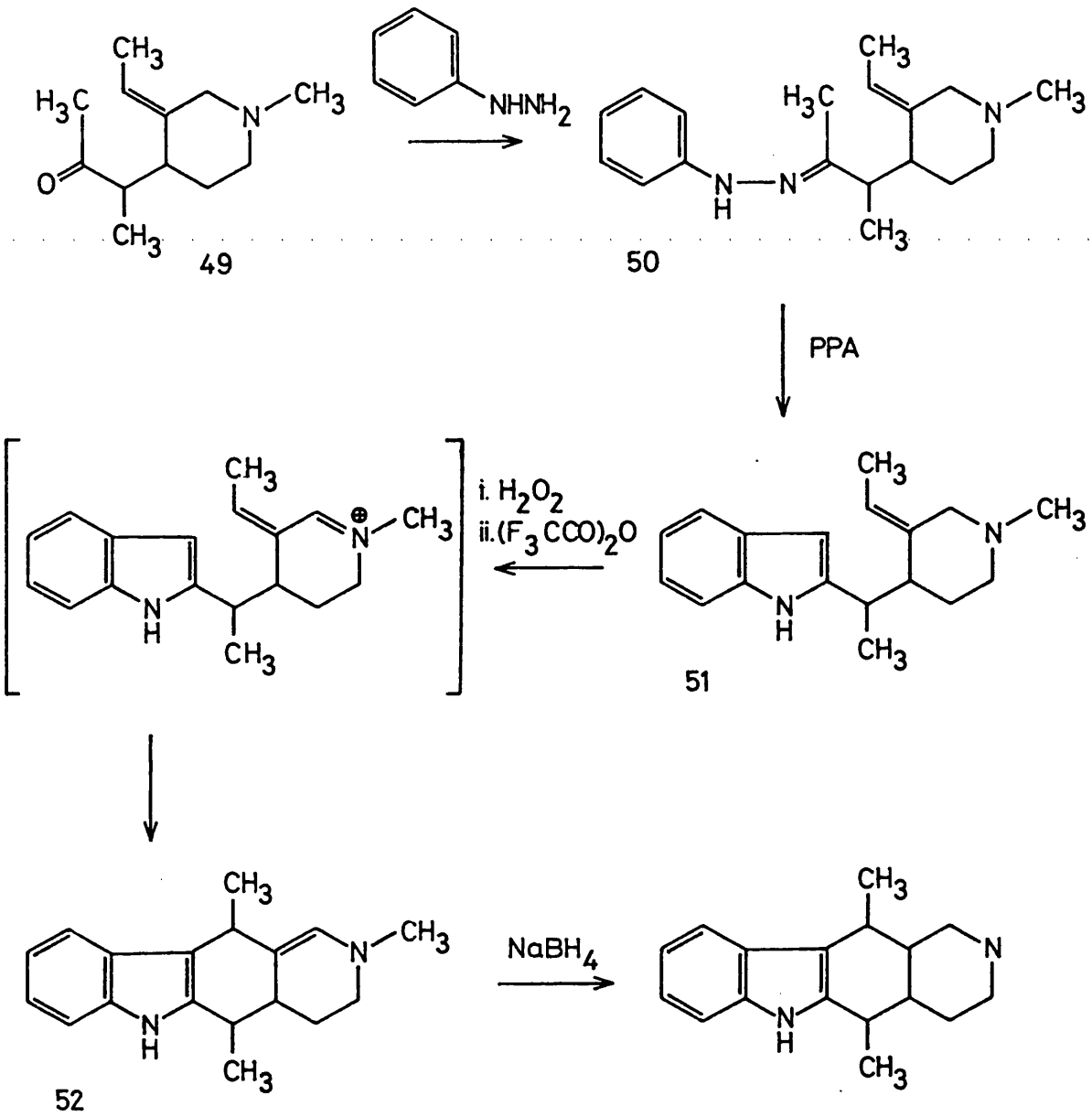
cyclisation to 2-methyl-1,2,3,4,5,11-hexahydroellipticine (52) which may be aromatised directly by heating with palladium on charcoal, or may be first reduced to the octahydro derivative using sodium borohydride prior to dehydrogenation. The yield of ellipticine by either method is about 20% from the phenylhydrazone (50).

Scheme 14



Once again these methods fall short of their synthetic potential because of the poor yields obtained in the final aromatisation step and, as mentioned previously, labile substituents are likely to be lost or changed under such severe conditions.

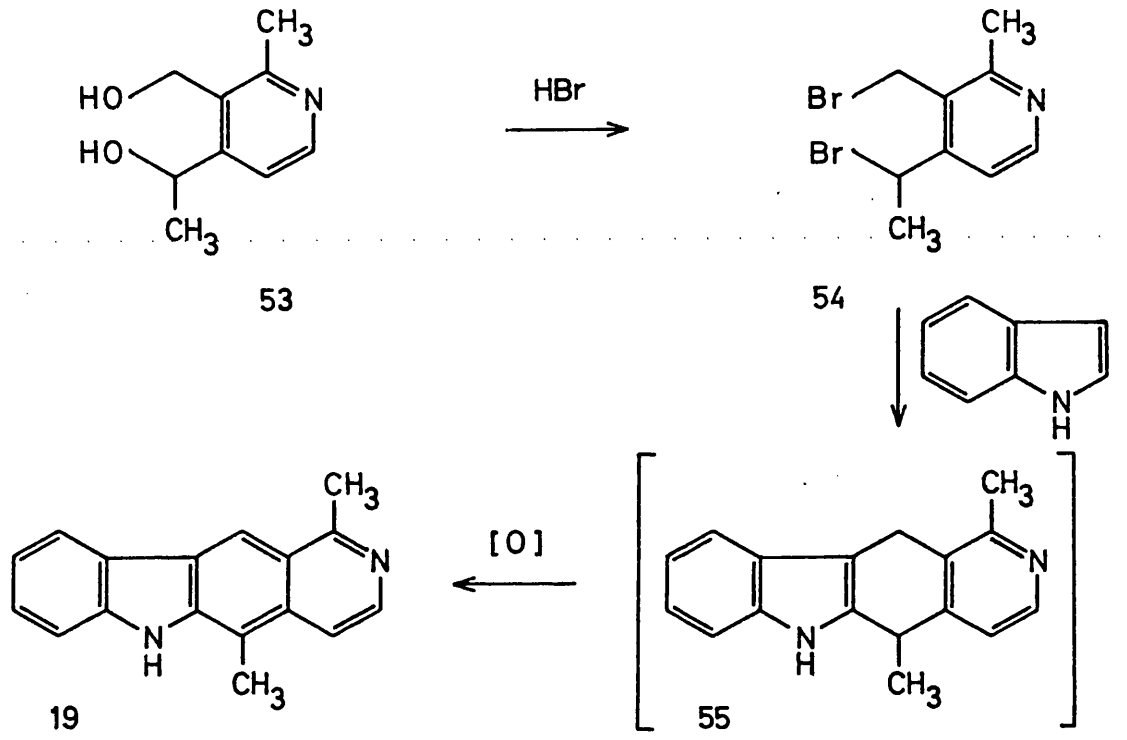
Scheme 15



49 50 51 52

A team of Japanese chemists led by Kametani[38] have devised a "one-step" synthesis of certain 6H-pyrido [4,3-b] carbazoles, although it should be pointed out that the starting material is itself the product of a multi-stage synthesis. In a typical example (see Scheme 16) 4-(1-hydroxyethyl)-3-hydroxymethyl-2-methylpyridine (53) is heated in 47% aqueous hydrobromic acid to give the corresponding dibromo compound (54) which is reacted with

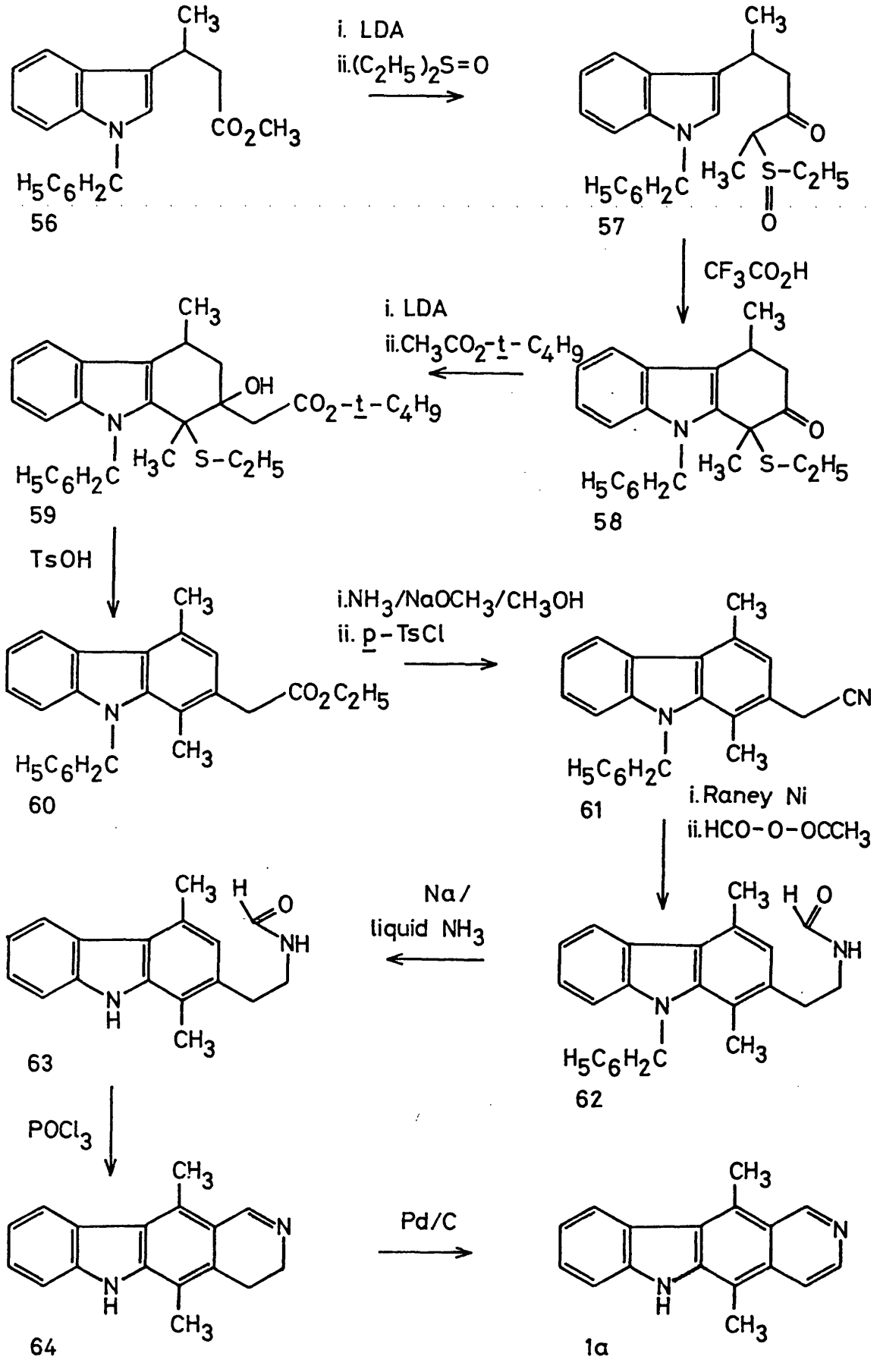
Scheme 16



indole without isolation of the intermediate to give olivacine (19) in 30% yield. Of special interest here is the regiospecificity of the reaction and the apparent ease with which the presumed intermediate dihydroolivacine (55) is oxidised to the fully aromatic structure.

In another effort by Japanese workers [39] ellipticine is synthesised from 3-[(1-benzyl)indolyl] methyl butyrate (56) in an overall yield of 23% (see Scheme 17). Treatment of the starting material (56) with the lithium salt of diethyl sulphoxide gives the sulphoxide (57) which is cyclised to (58) by heating with trifluoroacetic acid. The product, which is actually a mixture of diastereoisomers, is converted into (59) by treatment with tertiary-butyl lithioacetate in toluene. On heating with p-toluenesulphonic acid in a boiling mixture of xylene and ethanol this is converted to the carbazole derivative (60). This product is treated with

Scheme 17

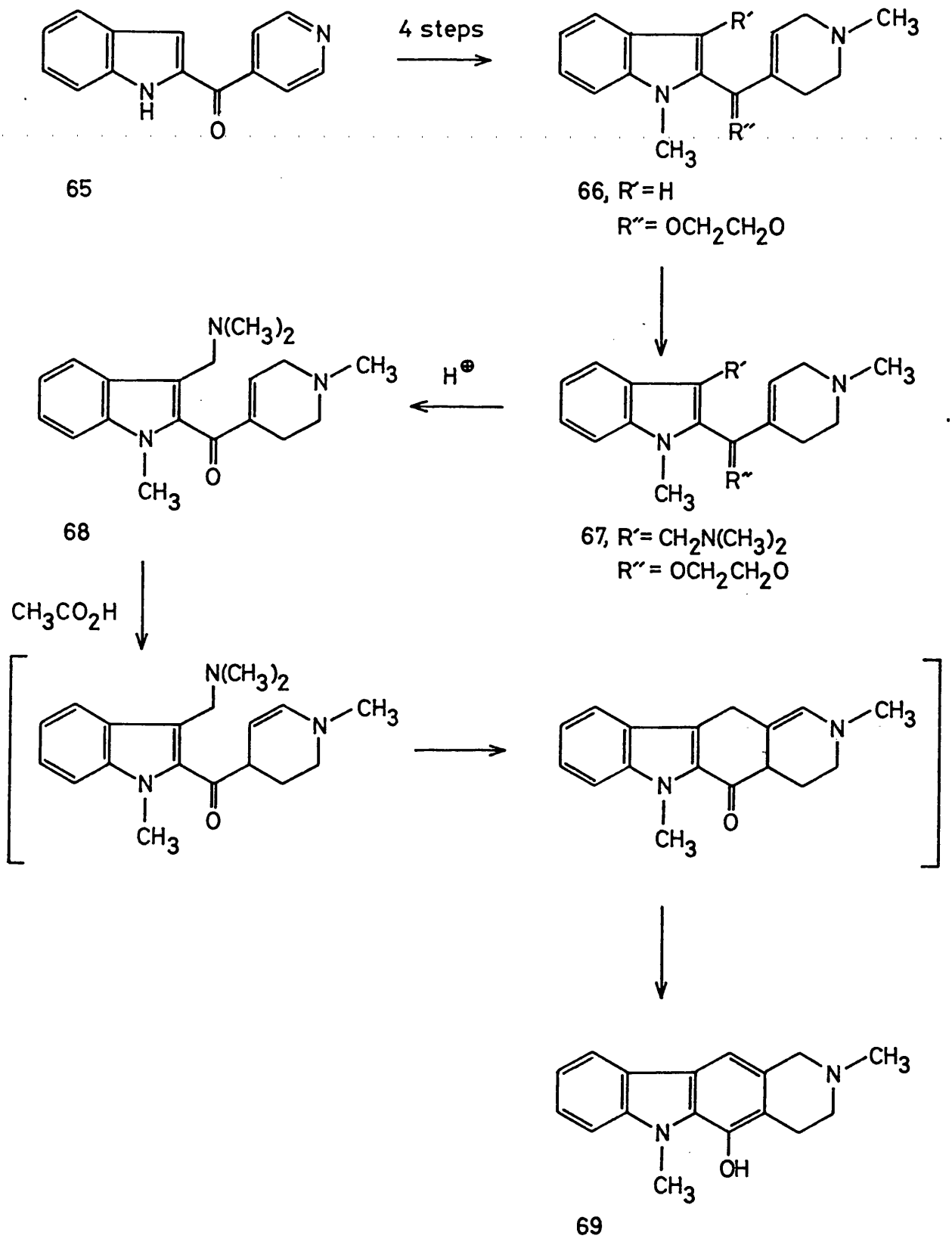

i. LDA
ii.(C2H5)2S=O
CF3CO2H
i. LDA
ii.CH3CO2-t-C4H9
TsOH
i.NH3/NaOCH3/CH3OH
ii. p-TsCl
i.Raney Ni
ii.HCO-O-OCCH3
Na/
liquid NH3
POCl3
Pd/C
56
57
58
59
60
61
62
63
64
1a

methanolic ammonia containing sodium methoxide to give an amide which is converted to the nitrile (61) by heating with p-toluene-sulphonyl chloride in pyridine. Catalytic reduction with Raney nickel and formylation using acetic formic anhydride gives (62) which is debenzylated with sodium in liquid ammonia to give the known compound (63)[26] (see Scheme 4). Bischler-Napieralski cyclisation is carried out using phosphoryl chloride in refluxing toluene to give 3,4-dihydroellipticine (64) which is heated with 10% palladium on charcoal to yield ellipticine.

Following the discovery that 9-hydroxyellipticine showed enhanced antitumour and antileukaemic activity[16] in comparison to ellipticine itself, Martinez and Joule[40] attempted to synthesise other hydroxylated derivatives. Their method of preparation of 5-hydroxy-2,6-dimethyl-6H-pyrido[4,3-b] carbazole (69) is outlined in Scheme 18. The key intermediate (66) is prepared from the pyridyl indolyl ketone (65) by protecting the carbonyl group through acetalisation, followed by N-methylation of the indole and pyridine units with subsequent reduction using sodium boro-hydride. This protected enone undergoes a Mannich reaction to give the indole 3-substituted product (67) which is then hydrolysed in 5M hydrochloric acid at room temperature to the unstable conjugated ketone (68). When this is refluxed in degassed 50% aqueous acetic acid, the desired pyridocarbazole is obtained, presumably *via* the indicated intermediates. However, once again the tetracycle is obtained in a reduced form which requires vigorous oxidation to give the fully aromatic ellipticine derivative.

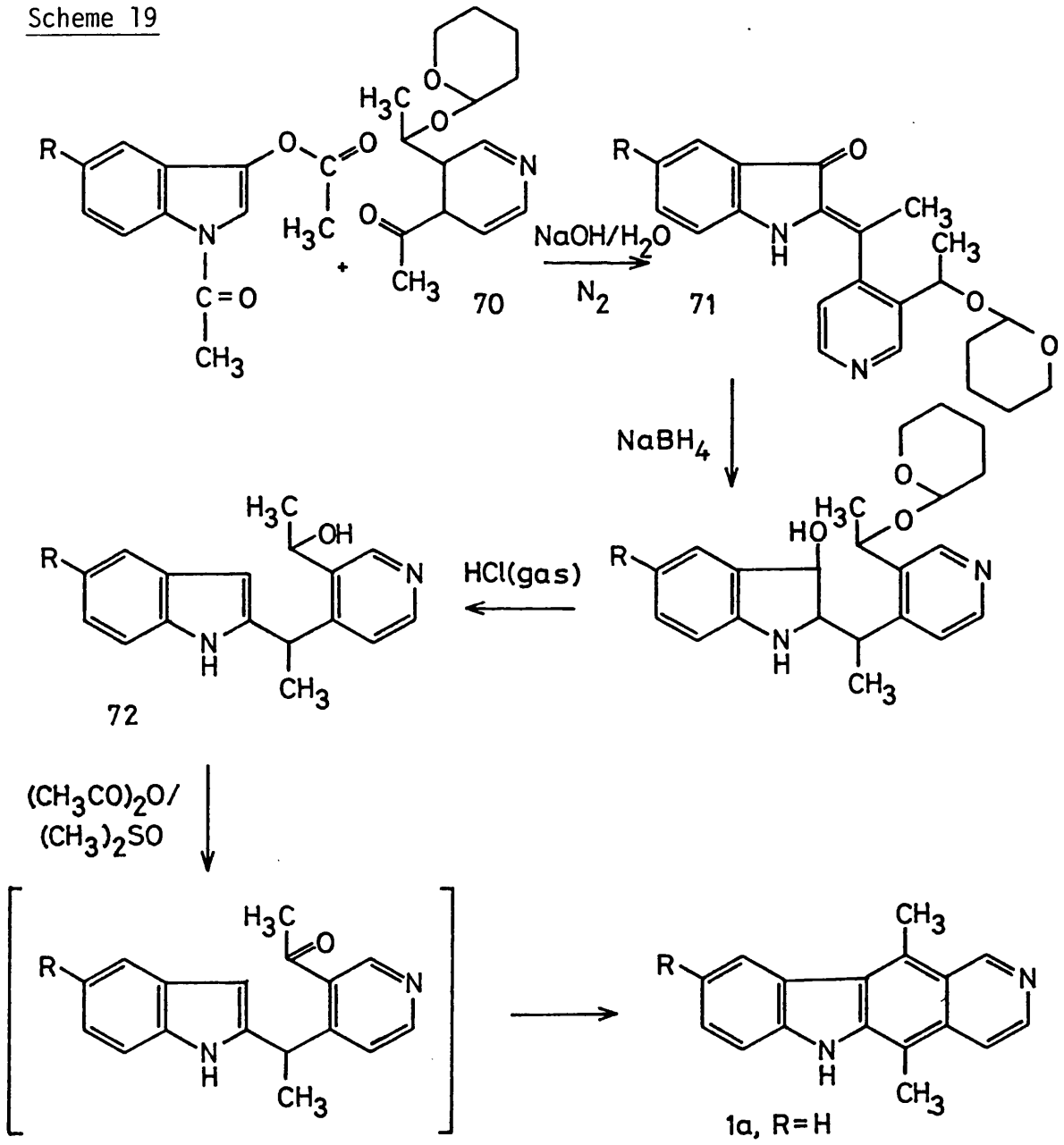
In an effort to overcome this problem, Sainsbury, Webb and Schinazi[41,42] devised a scheme using a suitable substrate at the correct oxidation level to form ellipticine directly upon ring

Scheme 18


4 steps
65
66, R′ = H
R″ = OCH2CH2O
67, R′ = CH2N(CH3)2
R″ = OCH2CH2O
H⊕
68
CH3CO2H
69

closure (see Scheme 19). Here 1-acetylindol-3-ol acetate or a 5-substituted derivative thereof, is condensed with the tetrahydropyranyl pyridine (70) to give the ethylideneindolin-3-one (71) as its E- and Z- isomers. The mixture is reduced with sodium borohydride, then treated with hydrogen chloride in chloroform to give the alcohol (72). Oxidation of this compound with acetic anhydride in dimethyl sulphoxide gives the acetyl derivative which immediately cyclises to ellipticine.

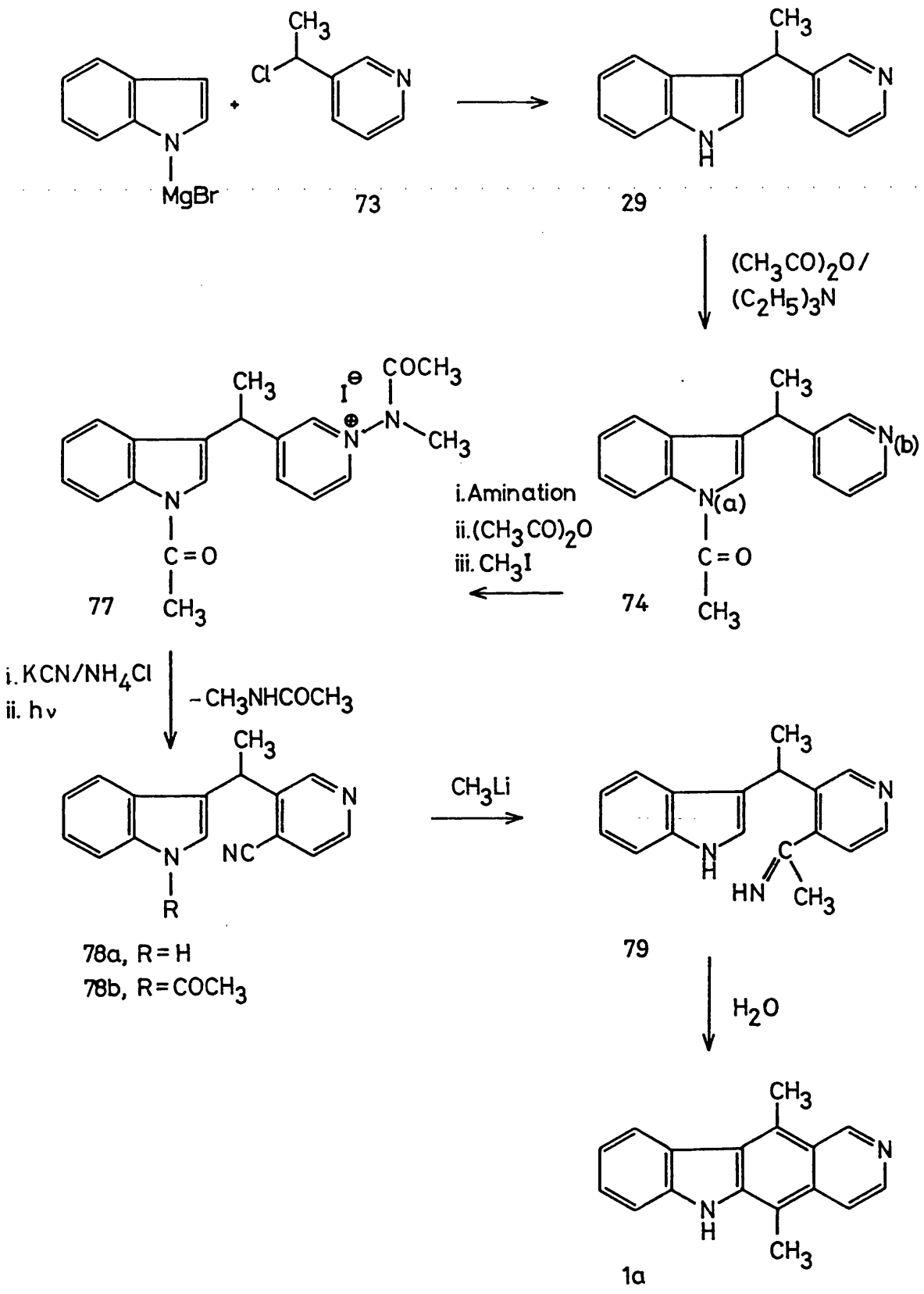
Scheme 19



Further developments of this work led to an entirely new route (see Scheme 20) incorporating an efficient preparation of 4-acetylpyridine derivatives which superseded the reductive acetylation technique used by Woodward in the original ellipticine synthesis. In this method indolylmagnesium bromide is reacted with 3-(1-chloroethyl) pyridine (73) to give 3-[1-(3-pyridyl)ethyl] indole (29). On treatment with acetic anhydride and triethylamine under reflux the $N_{(a)}$-acetyl derivative (74) is obtained which is treated with O-mesitylenesulphonyl hydroxylamine to form the amine salt. This is further treated with acetic anhydride and, without isolation, quarternised to the methiodide salt (77) by refluxing in excess iodomethane. In this form the 4-position of the pyridine nucleus is activated towards nucleophilic attack, whilst the 2- and 6-positions are effectively blocked by the substituent at the 1-position. Reaction with potassium cyanide under aqueous conditions gives the nitrile (78b) which may be de-acetylated by chromatography through a column of basic alumina using chloroform as eluent. Treatment with methyllithium gives the imine (79) which is hydrolysed with 20% aqueous acetic acid, resulting in spontaneous cyclisation and aromatisation to ellipticine. Subsequently, the scope and limitations of this method have been further explored by Sainsbury, Driver and Matthews[43,44].

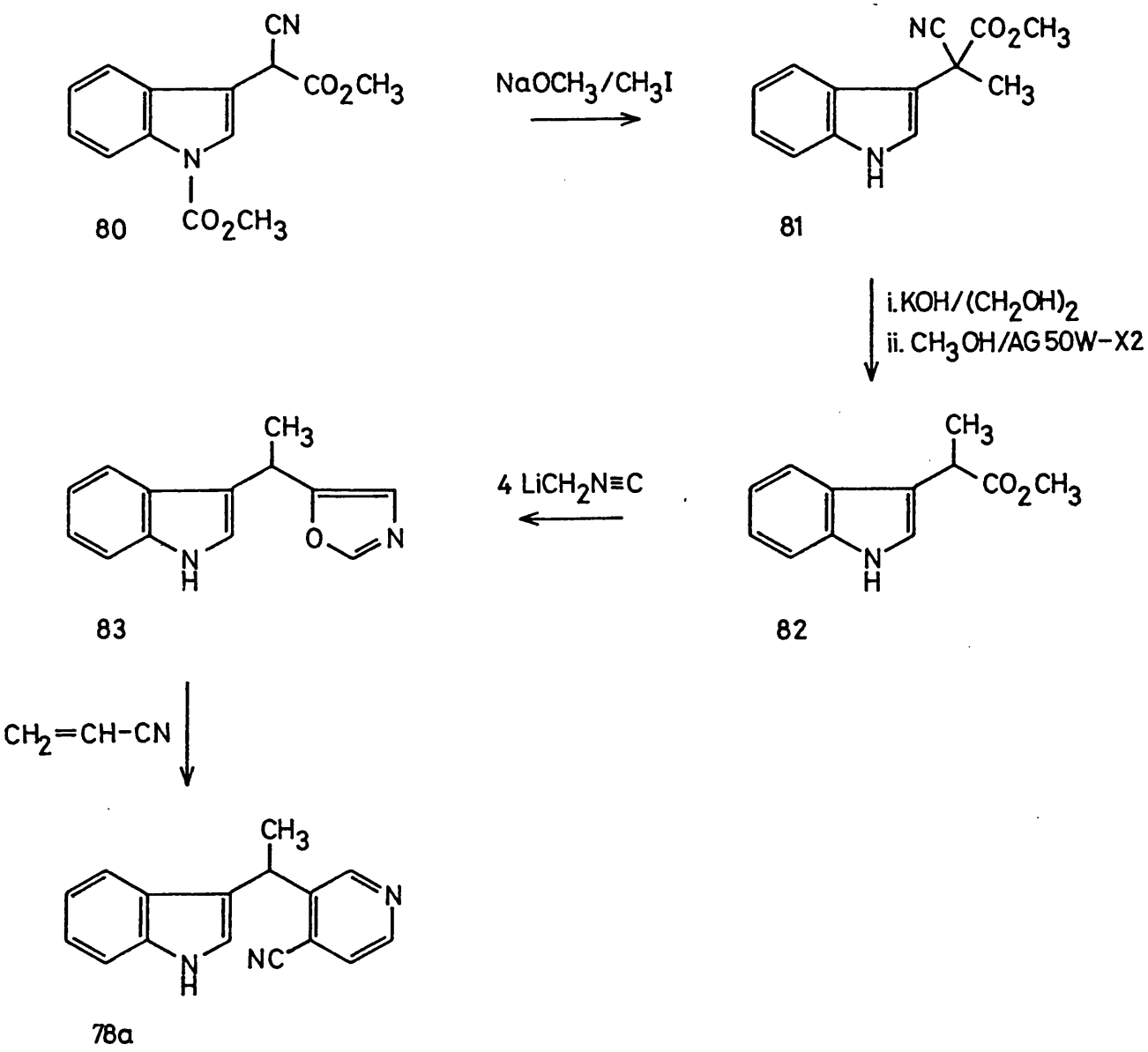
In early 1977, Kozikowski and Hasan[45] reported an alternative procedure for the preparation of the nitrile (78a) using a Diels - Alder reaction upon the oxazole (83) (see Scheme 21). The starting material for this sequence is prepared from gramine by the action of potassium cyanide and iodomethane, followed by dicarbomethoxylation using dimethyl carbonate and sodium methoxide in benzene. Further treatment with sodium methoxide and iodomethane results in the

Scheme 20


MgBr
73
29
$(CH_3CO)_2O$/
$(C_2H_5)_3N$
77
74
i. Amination
ii. $(CH_3CO)_2O$
iii. CH_3I
i. KCN/NH_4Cl
ii. hν
$-CH_3NHCOCH_3$
78a, R = H
78b, R = $COCH_3$
CH_3Li
79
H_2O
1a

removal of the N-carbomethyoxy group accompanied by C-methylation to give the product (81). On hydrolysis, decarboxylation and esterification this provides methyl-2-(3-indolyl) proprionate (82) which is reacted with excess α-lithiated methyl isocyanide to give the oxazole (83). This undergoes a Diels - Alder reaction with excess acrylonitrile in acetic acid to give 3-{1-[3-(4-cyano) pyridyl] ethyl} indole (78a) in 16% yield. Conversion of this compound into ellipticine is then achieved using the method of Sainsbury and Schinazi[42].

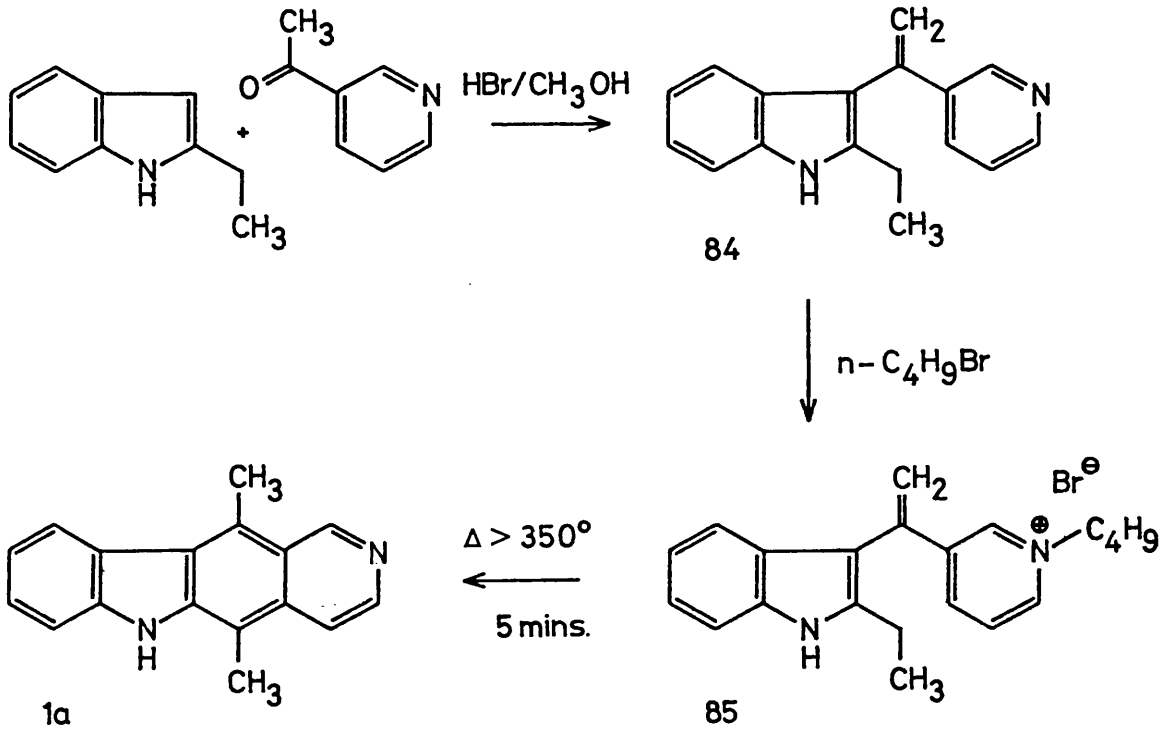
Scheme 21



The fact that this method, involving a crucial Diels - Alder reaction of low yield, is considered competitive is illustrative of the inaccessibility of ellipticine and its derivatives. This particular route opens up the possibility of preparing analogues containing peripherally modified D rings since the opportunity arises to vary either the α-metallated isonitrile or the dienophile employed in the Diels - Alder reaction.

Bergman and Carlsson[46] describe a very simple synthesis of ellipticine in which 2-ethylindole and 3-acetylpyridine are reacted to form the elusive 1:1 condensation product (84) (see Scheme 22). This is converted to the N-alkylated derivative (85) which is then pyrolysed for a short period at a very high temperature to give ellipticine in a claimed 72% yield. The severe conditions for this final step raise severe doubts as to the viability of this method for producing derivatives of ellipticine bearing labile substituents.

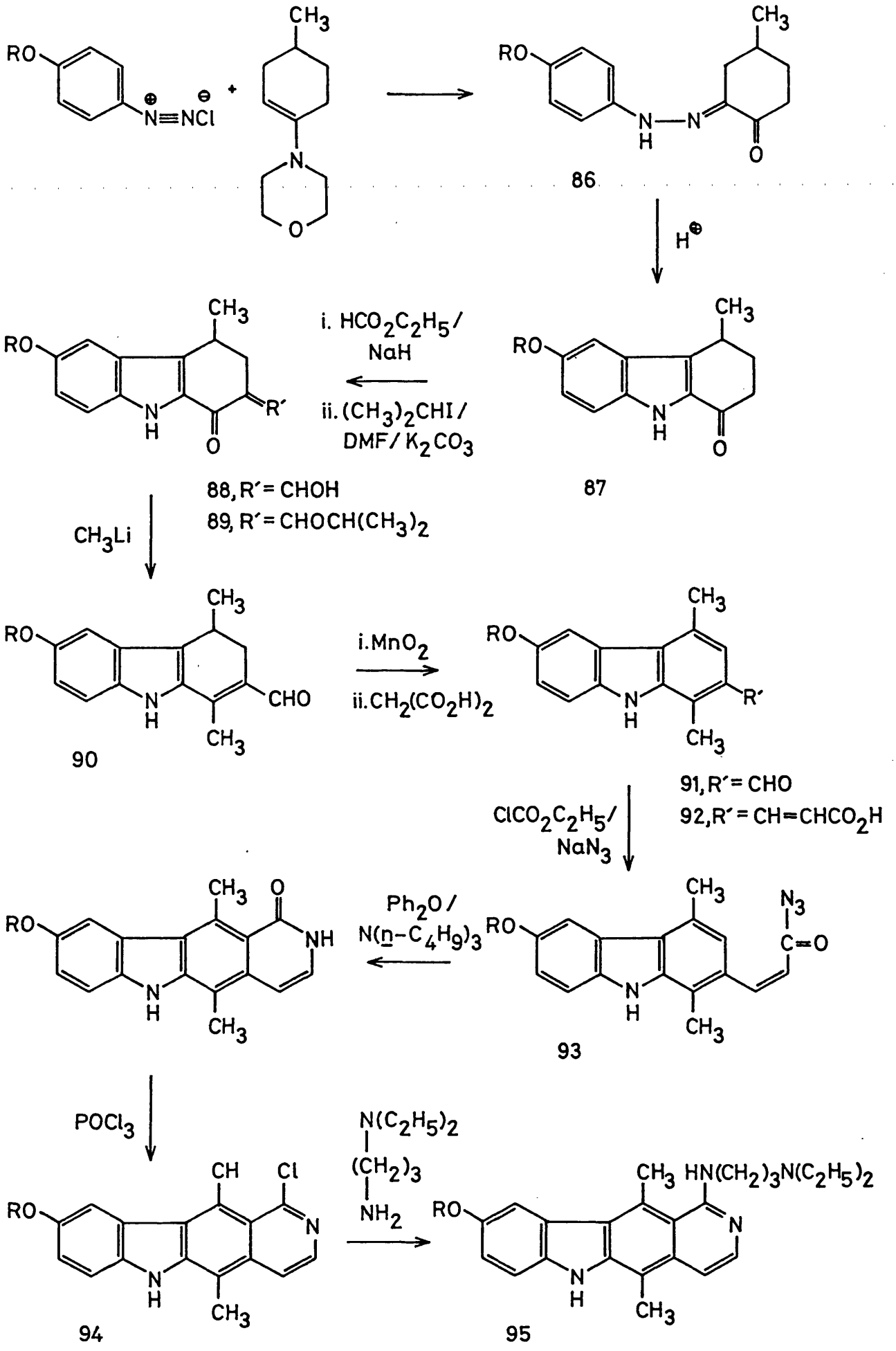
Scheme 22



Several interesting derivatives of ellipticine arose from the work of Bisagni's group in France[47], although it should be pointed out that this multi-step synthesis results in only modest yields of the alkaloid. Of particular interest is the inclusion of a dialkyl-amino-alkylamino side chain at the 1-position, which is expected to show enhanced antitumour activity against the L1210 leukaemia in mice[48]. In this method (see Scheme 23) 4-methoxybenzenediazonium chloride is reacted with 4-methyl-1-morpholinocyclohexene in dioxan to give the phenylhydrazone (86) which undergoes cyclisation under classical Fischer conditions. The dihydrocarbazol-1(2H)-one (87) is converted to the corresponding hydroxymethylene compound (88) by treatment with ethyl formate and sodium hydride. This is reacted with isopropyl iodide in dimethylformamide solution in the presence of potassium carbonate to give the isopropyl ether (89). Treatment with a large excess of methyllithium and subsequent hydrolysis results in the dihydrocarbazole (90) which is oxidised to the formylcarbazole (91) in a reaction with manganese dioxide. The aldehyde is condensed with malonic acid to give the acrylic acid (92) which is transformed into the azide (93) by treatment with ethyl chloroformate and sodium azide. Ring closure is carried out by boiling in diphenyl ether with tri-n-butylamine, and the chloroellipticine (94) is obtained by refluxing with phosphorus oxychloride. The final transformation into the 1-(3,3-diethyl-amino) propylamino derivative (95) is brought about by refluxing the chloro compound with the appropriate amine under an atmosphere of dry nitrogen.

A much less complicated synthesis of ellipticine has been reported by Taylor and Joule[49] in which the alcohol (96) (see Scheme 24) is oxidised to the ketone (97) with manganese dioxide, and is

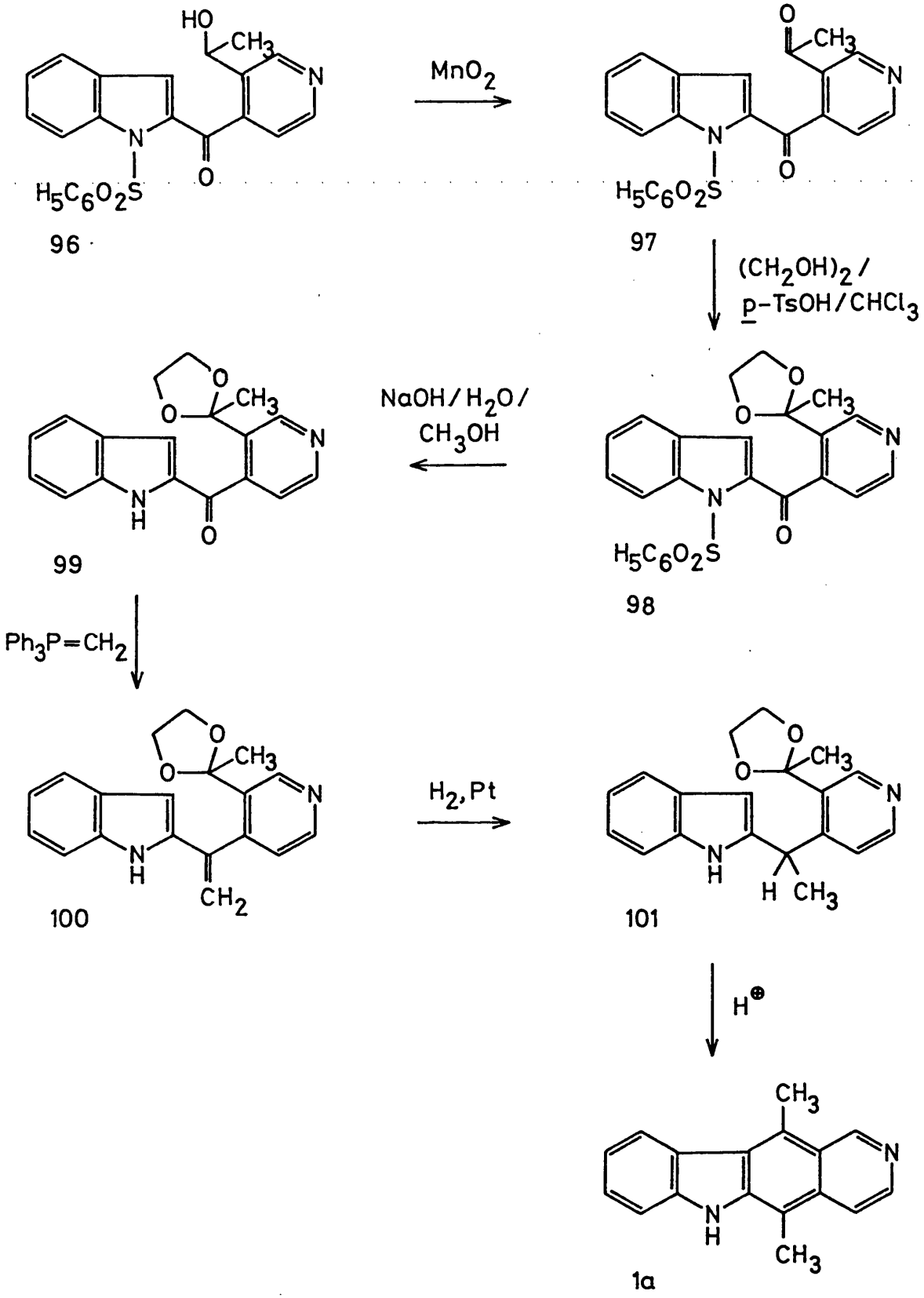
Scheme 23


RO
CH3
N≡NCl
N
O
86
H⊕
i. HCO2C2H5/ NaH
ii. (CH3)2CHI/ DMF/ K2CO3
R′
88, R′ = CHOH
89, R′ = CHOCH(CH3)2
87
CH3Li
CHO
90
i. MnO2
ii. CH2(CO2H)2
91, R′ = CHO
92, R′ = CH=CHCO2H
ClCO2C2H5/ NaN3
N3
C=O
93
Ph2O/ N(n-C4H9)3
NH
O
POCl3
CH
Cl
94
N(C2H5)2
(CH2)3
NH2
HN(CH2)3N(C2H5)2
95

then selectively acetalised using ethylene glycol and p-toluenesulphonic acid in refluxing chloroform. The keto-acetal (98) is debenzensulphonylated by alkaline hydrolysis and the new keto-acetal (99) is condensed with methylenetriphenylphosphorane in boiling tetrahydrofuran to give the alkene (100). Catalytic reductive conversion of this compound into the acetal (101) achieves the appropriate oxidation level and substitution pattern for the formation of ellipticine by treatment with dilute hydrochloric acid under reflux. This is a versatile method of preparation since it allows for the variation of the alkyl groups attached to the 5- and 11-positions. The nature of the C-11 alkyl group is determined at the beginning of the sequence since it arises in the starting material, whereas the C-5 alkyl group is introduced much later by means of a Wittig reaction.

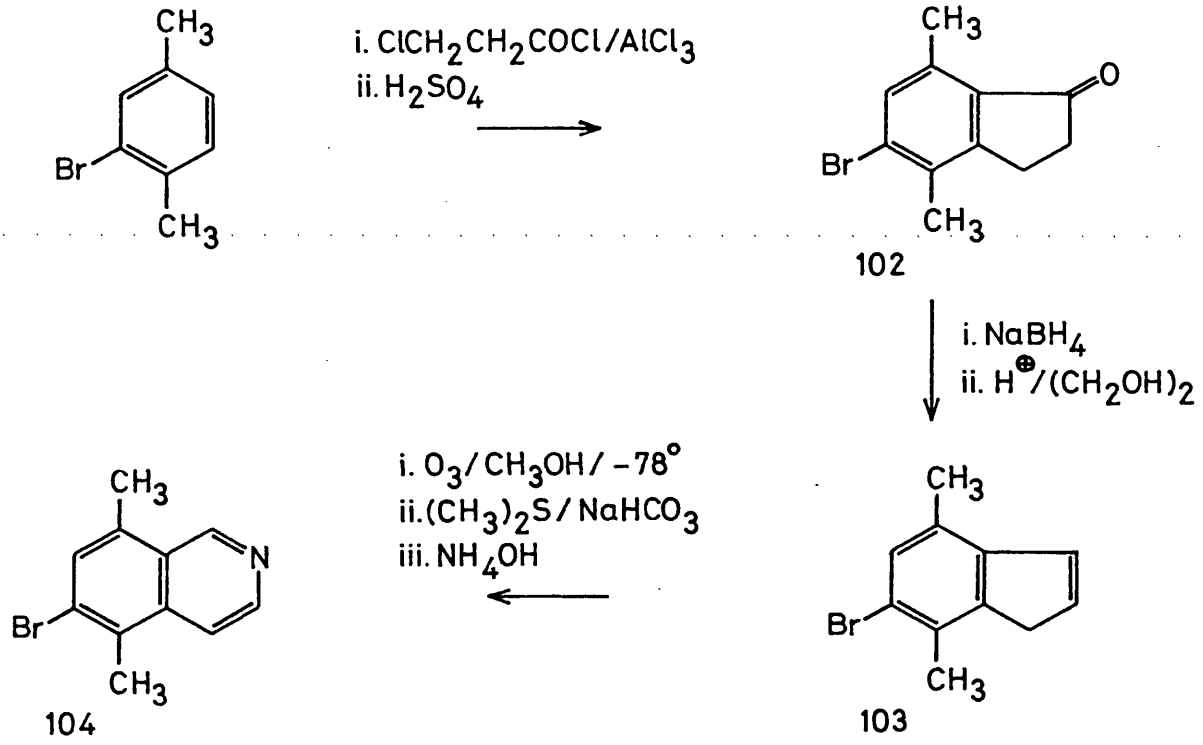
More recently Miller and Moock[50] have reported yet another synthesis which is quite unique in that the B ring of the parent alkaloid is formed in the last step, but not by the usual Fischer indolisation technique (see Scheme 25). Initially o-bromo-p-xylene is reacted with 3-chloropropionyl chloride in the presence of aluminium chloride, followed by treatment with sulphuric acid to give the indanone (102). This is reduced to the indene (103) by treatment with sodium borohydride followed by dehydration in boiling ethylene glycol containing 10% aqueous sulphuric acid. Ozonolysis of the indene gives an intermediate homophthalaldehyde which is reacted with ammonium hydroxide without prior isolation to give the desired 6-bromo-5,8-dimethylisoquinoline (104). This product is then coupled with an acetanilide to give the diaryl amide (105) which is hydrolysed under acid conditions to the

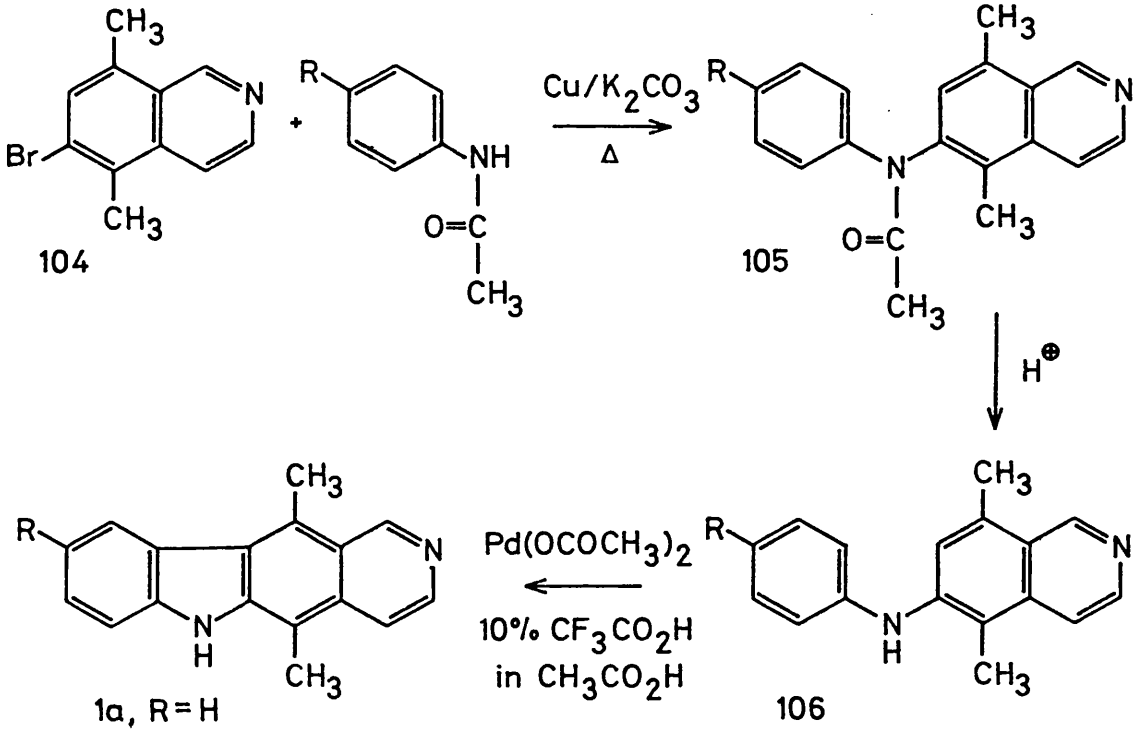
Scheme 24


HO
CH3
N
MnO2
O
CH3
N
H5C6O2S
O
96
97
(CH2OH)2 /
p-TsOH/CHCl3
NaOH/H2O/
CH3OH
O
CH3
O
N
H
99
98
Ph3P=CH2
H2,Pt
CH2
100
H CH3
101
H⊕
CH3
1a

aryl isoquinoline amine (106). This product does not undergo photochemical ring closure, but cyclisation may be effected using one or two molecular equivalents of palladium acetate in a mixture of trifluoroacetic acid and acetic acid. The authors claim only a modest yield of 25% for the final step,but the entire sequence is nevertheless reckoned to be competitive with other routes. Moreover, an attempt is made to maximise its flexibility by keeping the A ring and the isoquinoline moiety separate until the latter stages of the synthesis.

Scheme 25


i. ClCH2CH2COCl/AlCl3
ii. H2SO4
i. NaBH4
ii. H⊕/(CH2OH)2
i. O3/CH3OH/ -78°
ii.(CH3)2S/ NaHCO3
iii. NH4OH
102
103
104


Cu/K2CO3
Δ
H⊕
Pd(OCOCH3)2
10% CF3CO2H
in CH3CO2H
104
105
106
1a, R=H

DISCUSSION

DISCUSSION

Preparation of 3-[1-(3-Pyridyl)ethyl] indole (29):

Whilst it is true that the synthesis of 6H-pyrido [4,3-b] carbazole derivatives via 3-[1-(3-pyridyl) ethyl] indoles is a versatile method,[43,44] problems still remain to be solved in the preparation of the starting materials. In the past this was carried out by condensing two equivalents of indolylmagnesium bromide (from indole and ethylmagnesium bromide) with one equivalent of 3-(1-chloroethyl) pyridine (73) in diethyl ether at -10^{o}, according to the method of De Graw et al[51]. The resulting mixture was allowed to warm slowly to room temperature and was stirred for two days before extracting with ice-cold dilute hydrochloric acid. Over a series of such preparations the yield of 3-[1-(3-pyridyl) ethyl] indole averaged only 21%.

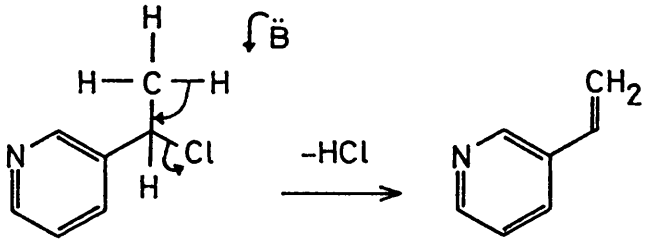
A significant improvement in this figure was achieved when the ethereal suspension of indolylmagnesium bromide was homogenised by the addition of a little dichloromethane and when the product was exhaustively extracted with hot dilute hydrochloric acid. Under these conditions the yield could be increased to 24%.

The length of time during which the reaction mixture was left stirring also affected the yield. In an effort to establish the relationship here, several experiments were conducted in which the stirring time was varied with all other parameters remaining constant. If it was desired to prepare 3-[1-(3-pyridyl)ethyl] indole in a single day then it became necessary to cut the stirring time down to about two hours. Under these circumstances the isolated yield of the product was a meagre 3%. Even after twenty-four hours the product yield was still some way short of the optimised value at 11%.

Longer periods of stirring did not appear to enhance the yield significantly, even when the experiment was prolonged for a week, but neither was any diminution observed.

This would seem to indicate that the reaction is an equilibrium process, and explains why the product yield increases when two mole-equivalents of indolylmagnesium bromide are used[52], in accordance with the Law of Mass Action.

The nature of the tarry residue obtained when the reaction mixture is stirred with hydrochloric acid has never been fully investigated. It is assumed, however, that it is largely composed of polymeric material derived from 3-vinylpyridine and the hydrolysis products of the unconsumed indole Grignard reagent. 3-(1-Chloroethyl) pyridine is thermally unstable and, if prepared in advance, must be stored in a refrigerator below -5^{o}. Indeed, attempts to prepare the corresponding bromo compound have proved futile because it decomposes by dehydrohalogenation as soon as it is isolated. In all probability the loss of hydrogen chloride from 3-(1-chloroethyl) pyridine is hastened under the conditions employed here because of the basicity of the Grignard reagent, viz:



Recent work in this laboratory[53] has led to a further improvement in the preparation of 3-[1-(3-pyridyl)ethyl]indoles so that these compounds are now available in yields of up to 30%. This has been achieved by carrying out the reaction in freshly-distilled dry

tetrahydrofuran, rather than anhydrous diethyl ether. There is much evidence to suggest that the choice of solvent is very important in Grignard reactions[54,55], reflecting the necessity to solvate the various species present, for they are not simply alkyl- or arylmagnesium cations and attendant halogen anions[56,57]. Even today there is some doubt about the structure of Grignard reagents: Although they are commonly written as RMgX, where X is chlorine, bromine or iodine, it is probable that they exist in equilibrium with di-organomagnesium and magnesium halide, as shown below:

$$2RMgX \rightleftharpoons R_2Mg + MgX_2$$

Tetrahydrofuran is a stronger base than diethyl ether, and can more readily form coordination complexes with the organometallic species in solution. This has the effect of removing such compounds from the metal surface as soon as they are formed, thereby exposing a new surface for further reaction. It has been suggested that the enhanced basicity of tetrahydrofuran results from the greater accessibility of the lone pairs of electrons on the oxygen atom. By strongly solvating the organometallic species, tetrahydrofuran effectively shifts the equilibrium in favour of RMgX and this aids better subsequent reaction.

If it is assumed that the reaction of the pyridyl halide is of the S_N1 type, by analogy to the benzyl halides, then two competing processes may be envisaged:

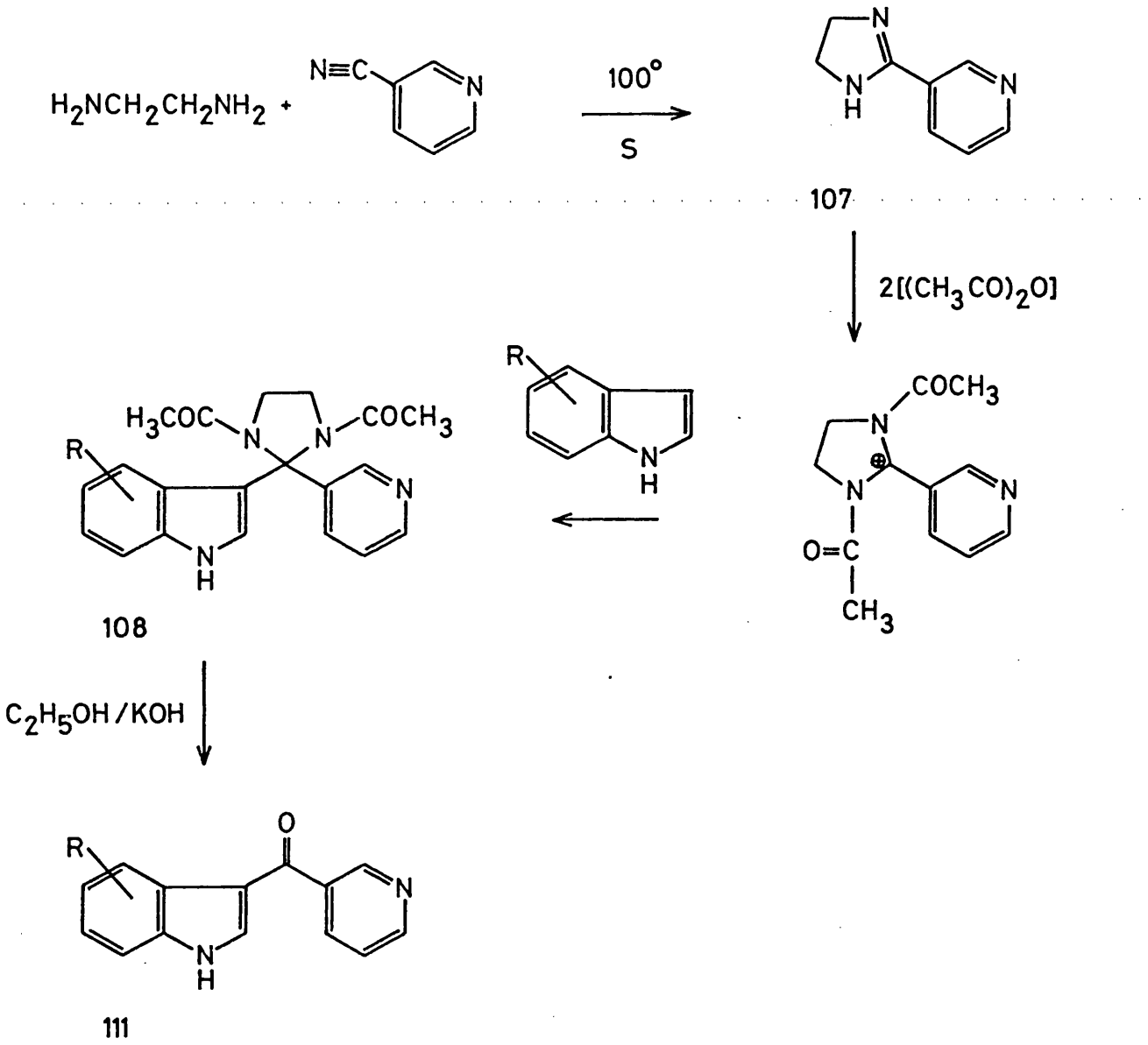
a. Normal reaction at the indole 3-position to afford the required product, or

b. Elimination of a proton from the cationic species to yield 3-vinylpyridine which may then polymerise.

In an effort to find an alternative preparation of 3-[1-(3-pyridyl)ethyl] indole, attention was focused on the ketone, indol -3yl-3-pyridyl methanone (111). Previous work in this laboratory[43] has shown that it is possible to prepare this compound in 60% yield from the condensation between indolylmagnesium bromide and nicotinoyl chloride at low temperature. However, one of the disadvantages of this method is that in order to maximise the yield of the ketone, a 1.5 molar excess of indole is required[58]. Although this is more favourable than the two mole-equivalents of indole needed for the reaction with 3-(1-chloroethyl) pyridine, a method was sought which would be still more economical with respect to indole. This would enable ellipticine derivatives bearing substituents in the A ring to be more readily synthesised.

Fortunately an interesting technique became available[59] in which the pyridine moiety was derived from a readily available starting material, in this case 3-cyanopyridine. This compound was reacted with 1,2-diaminoethane by heating in the presence of a catalytic amount of sulphur to give the dihydroimidazole(107). This was stirred briefly with acetic anhydride and then one molar equivalent of the desired indole was added with cooling to give the imidazoline (108). Hydrolysis was achieved by treatment with ethanolic potassium hydroxide and the product was recrystallised from ethanol in excess of 70% with respect to the indole. The reaction scheme is outlined over.

It should be noted here that the product (111) is not a true ketone but a vinylogous amide and hence its reactivity towards nucleophiles is difficult to predict. Nevertheless, in what was expected to be a straightforward reaction, indol-3-yl-3-pyridyl methanone (111) was dissolved in anhydrous tetrahydrofuran

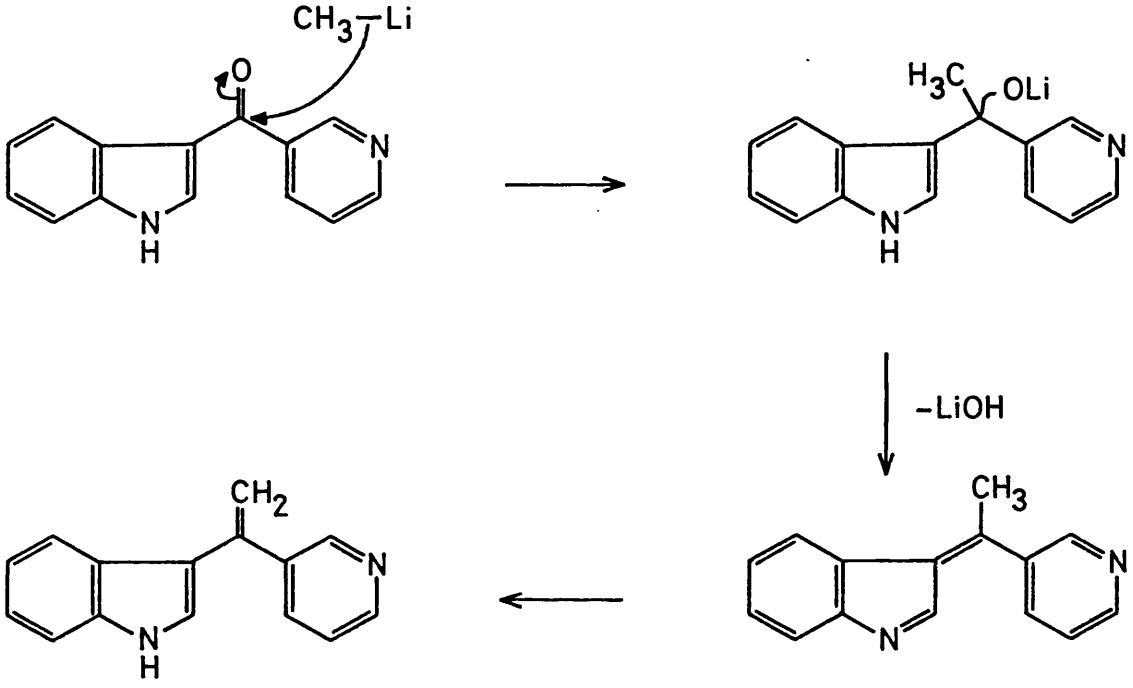


and treated with a solution of methyllithium in hexane. The initial experiment was conducted at -10°, allowing the reactants to warm up overnight.

The isolated product was an impure, low-melting solid which exhibited two major spots on TLC in 2% methanol in chloroform. Neither of these spots corresponded to the starting material and repeated attempts at recrystallisation from a variety of solvents

proved futile. Chromatographic separation was also unsuccessful because the eluted material was invariably the same as the initial mixture. A second experiment conducted at -78^{o} also yielded the same mixture of products.

The expected product of this reaction was the known compound, 1-(indol-3-yl)-1-(3-pyridyl)ethene (110)[46,60]. The reaction is presumed to proceed via an intermediate formed by initial attack at the carbonyl centre by methyllithium:



110

Satisfactory ^{1}H nuclear magnetic resonance data could not be obtained for the isolated material, but infra red and mass spectra were recorded. The mass spectrum shows the parent molecular ion at m/e 220, which is two mass units less than the starting ketone. However, the fragmentation pattern does not compare well with the data recorded by the Swedish team for pure (110). No further comparison was possible since the Swedes did not report an infra red spectrum. The product isolated at Bath exhibits a band at 3150cm^{-1},

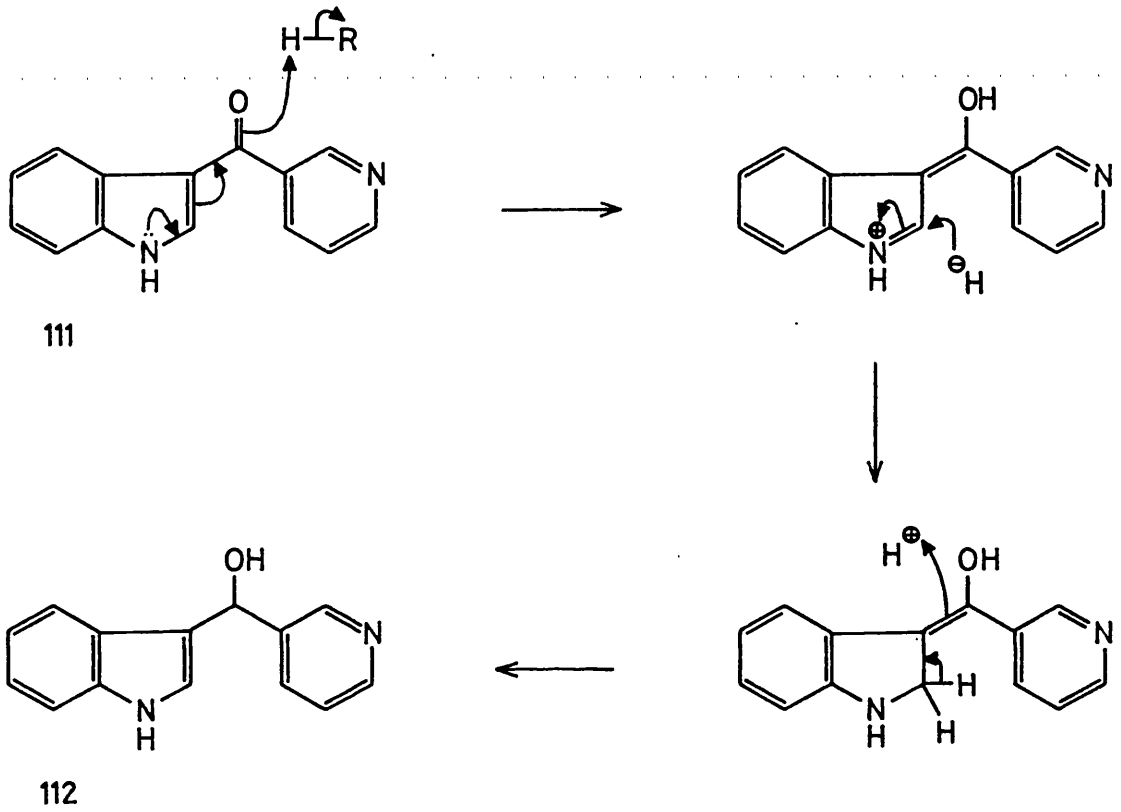
indicating that the indole N-H is retained, and also has ν_{max}=1620cm^{-1} which is characteristic of the aryl-conjugated vinylic C=C stretch.

In view of the difficulty encountered in obtaining satisfactory spectral data, it was decided that the best way to identify this product would be to carry out the subsequent reaction step, which was a reduction process.

Treatment with sodium borohydride in ethanol was the most fruitful approach, but an investigation of the reduction product by TLC indicated that the isolated material was a complex mixture and attempts to purify it by recrystallisation only slightly improved its purity. Column chromatography proved equally unproductive.

Lithium aluminium hydride did not appear to react with this material as the TLC evidence showed the reaction product to be identical with the starting compound. Hydrogenation under a pressure of 100 p.s.i. using platinum oxide catalyst was also attempted, but no change took place.

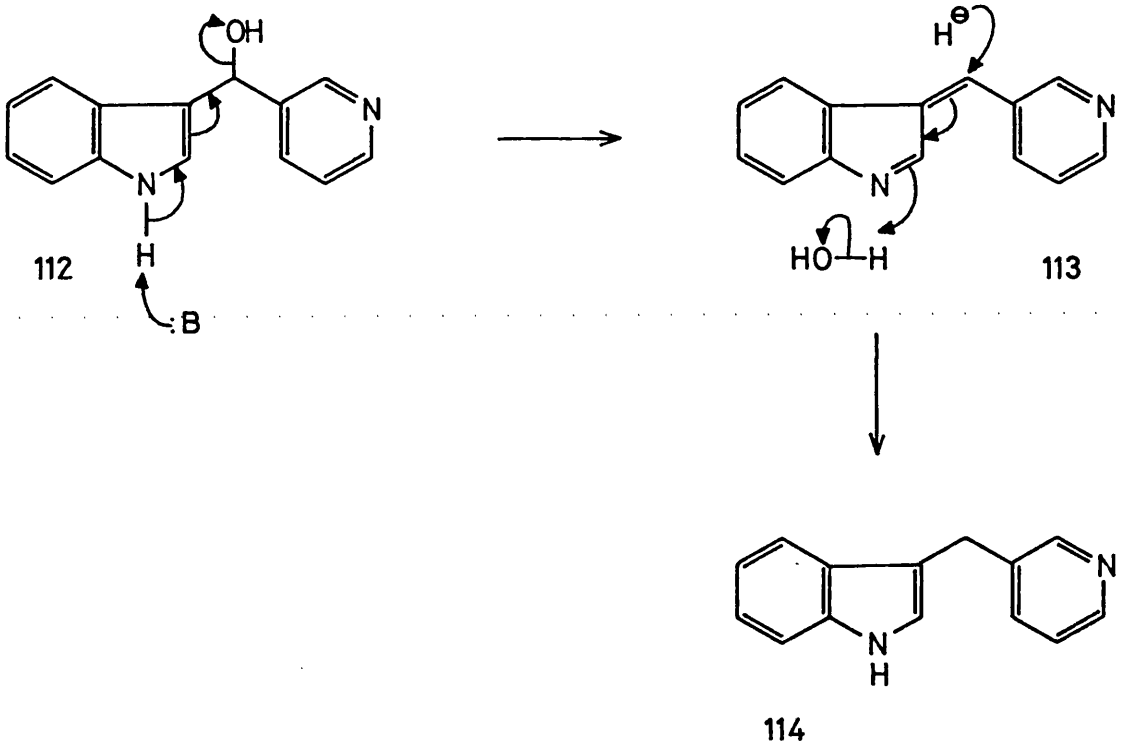
At this stage it was decided to attempt a slight modification of this approach, using a different substrate. Interestingly, when the ketone (111) was treated with sodium borohydride in ethanol at temperatures below 10^{o}, the alcohol (112) was formed, whereas a corresponding reaction with lithium aluminium hydride in anhydrous tetrahydrofuran returned unchanged starting material. Clearly, since both of these reagents act as vigorous nucleophiles, the observed difference in reactivity must be due to the solvent. Lithium aluminium hydride is used in aprotic solvents so it would seem reasonable to suggest that the formation of the alcohol from (111) might therefore involve initial O-protonation in an aqueous medium. This is followed by reduction of the resultant iminium salt, as indicated over:



111

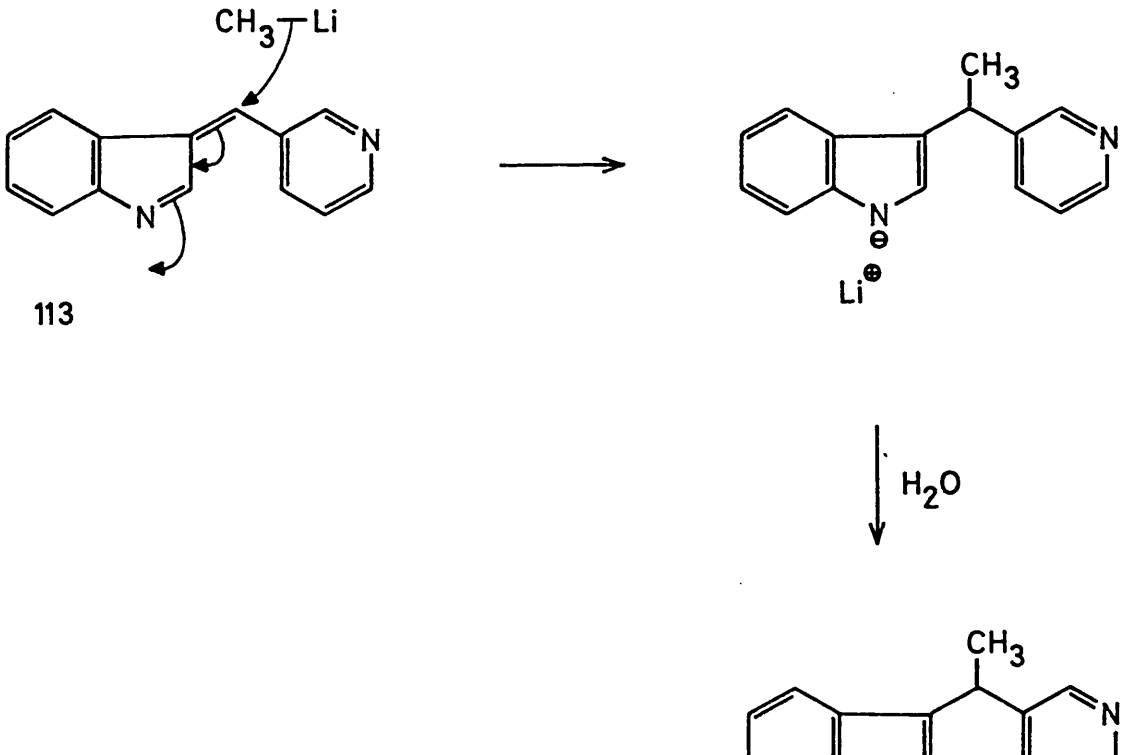
112

With excess sodium borohydride at higher temperatures indol-3-yl -3-pyridyl methane (114) was formed. This reaction may be rationalised by postulating the base-catalysed dehydration of the alcohol (112) to the indolenine (113) which then undergoes further reduction, as shown on the following page:



112 113 114

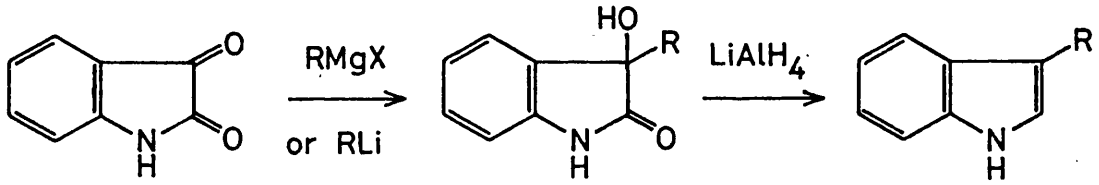
In view of this it was hoped that the alcohol might react with an excess of methyllithium, firstly to form the indolenine, and subsequently the required compound (29):



113 29

In practice, however, reaction of the alcohol with methyllithium under various conditions gave only complex mixtures which could not be characterised.

Yet another approach to the problem of synthesising 3-[1-(3-pyridyl)ethyl] indole (29) in acceptable yield involved the conversion of isatin (115) into a 3-substituted indole such as (117) via a 3-hydroxyoxindole derivative (116):

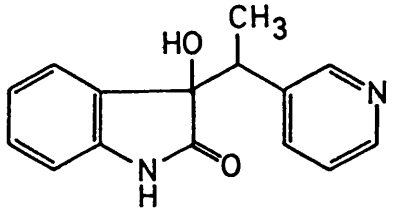


115 116 117

Previously this method has been seldom used[61-63] since it is considered to be somewhat time-consuming and the overall yields are unsatisfactory. However, some of these disadvantages have been partly overcome by integrating the steps[64], so that the 3-hydroxyoxindole derivative is reduced directly without isolation.

Following Bergman's successful preparation of 3-(4-pyridyl) indole by this method, attempts were made to prepare the corresponding Grignard reagent of 3-(1-chloroethyl) pyridine (73) and react this with isatin. Unfortunately the halo-pyridine resisted all efforts to form the Grignard reagent, remaining unchanged even when iodine was added to initiate the reaction and when the reaction mixture was warmed to reflux on a water bath. A second attempt was made by preparing the lithium organometallic derivative of the pyridylethyl chloride and this proved to be much more successful. Addition of isatin and subsequent treatment with lithium aluminium

hydride gave an orange oil which yielded a buff-coloured solid on trituration with petroleum ether. However, investigation of this compound indicated that the desired 3-[1-(3-pyridyl)ethyl] indole had not been formed and it seemed most likely that the intermediate 3-hydroxy-3-[1-(3-pyridyl)ethyl] oxindole (118) had been produced instead:

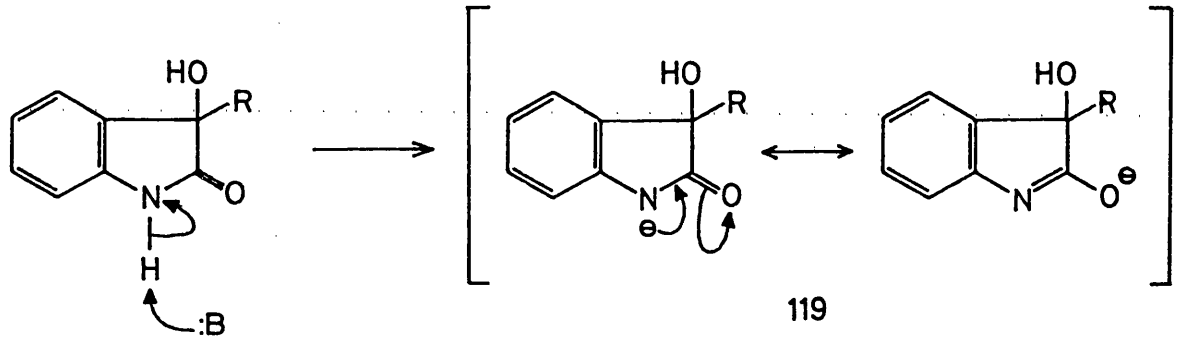


118

Regrettably a satisfactory ^{1}H nuclear magnetic resonance spectrum could not be obtained for this material, owing to the presence of paramagnetic impurities. The compound could not be recrystallised and it was only possible to obtain a solid sample by using the trituration procedure mentioned previously. However, in its infra red spectrum the compound displayed a very broad peak at 3260cm^{-1} which is characteristic of a hydroxyl group, and only a single carbonyl absorption at 1715cm^{-1}. The mass spectrum showed a very weak molecular ion at m/e 254 corresponding to the structure (118).

This result was not altogether surprising since oxindoles are notoriously difficult to reduce, as previous work in this laboratory has demonstrated [65]. It is known, for instance, that lithium aluminium hydride is an effective reagent for reducing N-alkylated oxindoles to indoles[63], but it is widely accepted that unsubstituted compounds are resistant to reduction by this reagent.

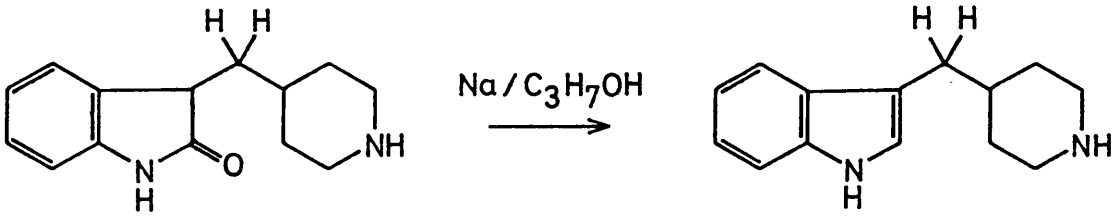
Presumably this is because the anion (119) is formed in the presence of a strong base, and this species is resistant to further attack by nucleophilic reagents such as lithium aluminium hydride:



119

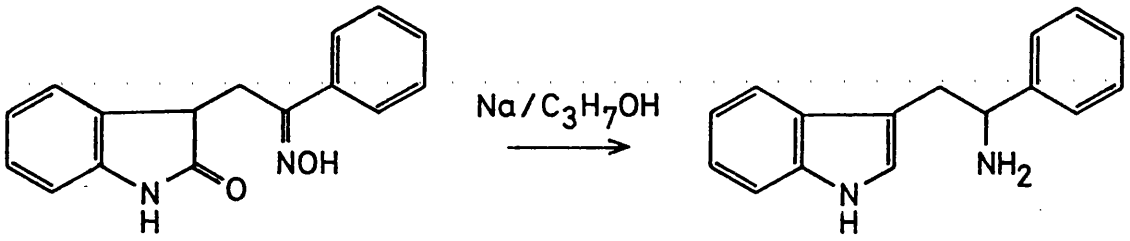
The earliest example of the reduction of an oxindole was reported by Baeyer[66] over one hundred years ago, but in this case the oxindole vapour was passed over hot zinc. Clearly this method is of limited synthetic value when applied to complex derivatives.

Sodium in alcohol is also said to reduce oxindoles, but here again there is much evidence to suggest that only highly alkylated derivatives are reduced to indoles[67]. Using this technique, however, a team of Italian workers has effected the conversion of the oxindole (120) to the indole (121) by heating with sodium in boiling propan-1-ol[68]:



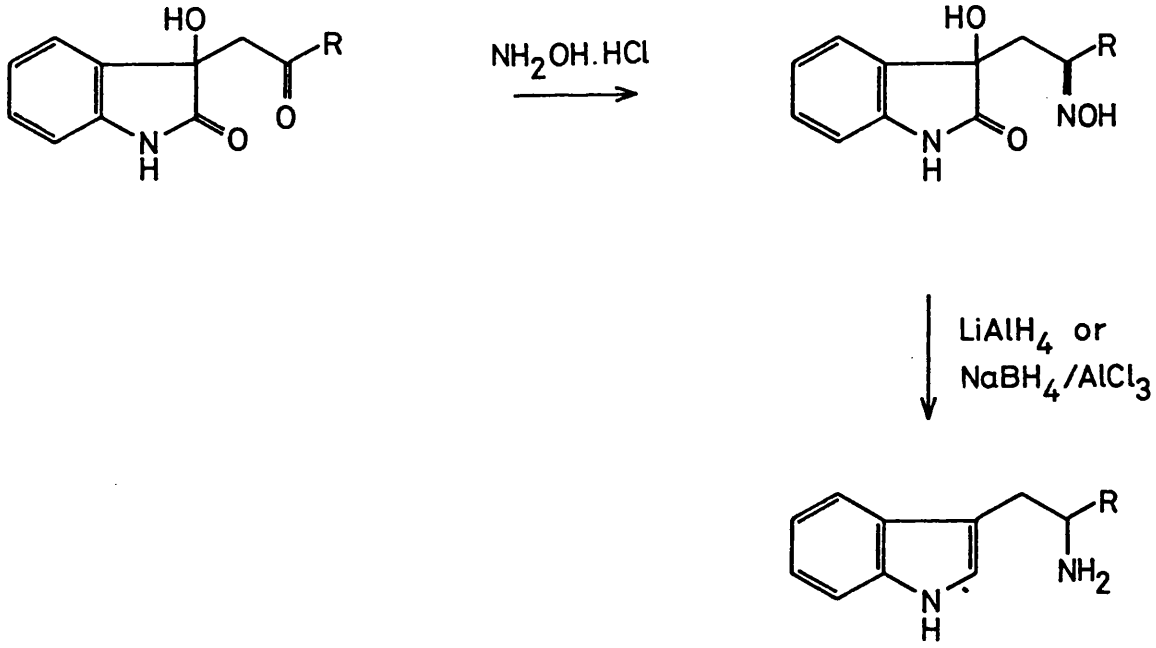
120 121

An earlier paper[69] describes the preparation of α-phenyltryptamine (123) from isatylideneacetophenone oxime (122) using the same combination of reagents:

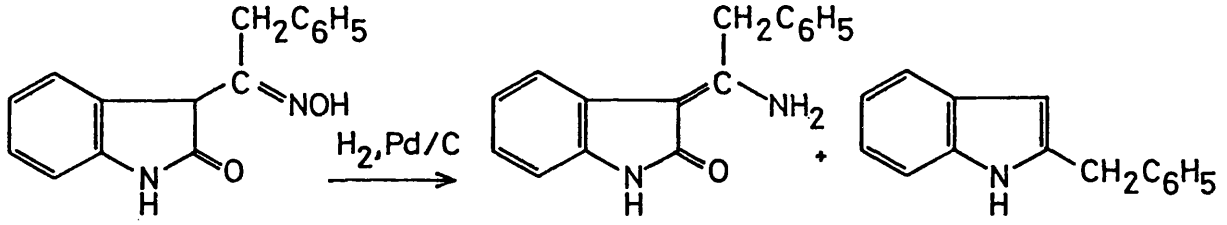


122 123

A similar procedure has also been used by Franklin and White[63] to prepare other α-substituted tryptamines from isatin condensation products. Here, however, reduction of the oxindole was achieved by treatment with lithium aluminium hydride in tetrahydrofuran or alternatively by the sodium borohydride/aluminium chloride complex:

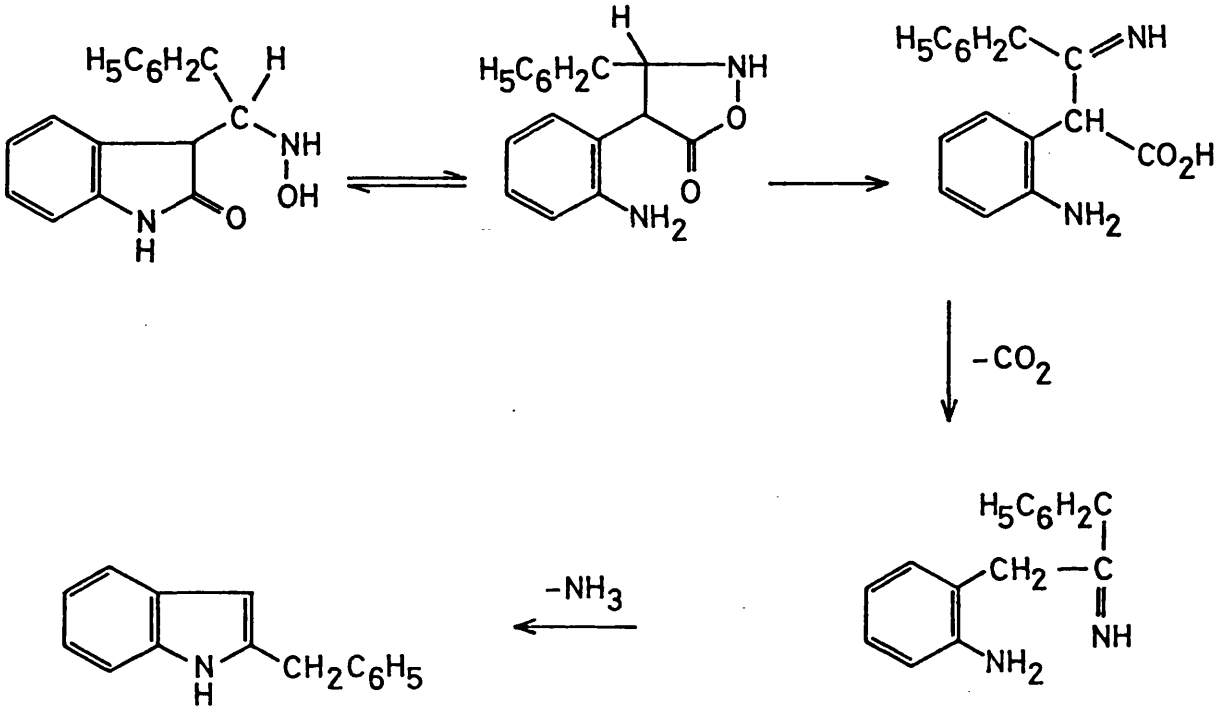


Wenkert and co-workers[70] observed that catalytic hydrogenation of similar 3-substituted oxindole oxime derivatives resulted in the formation of 2-substituted indoles as well as the expected products. For instance, with 3-phenylacetyloxindole oxime (124) a mixture of 3-(1-amino-2-phenylethylidene) oxindole (125) and 2-benzylindole (126) was obtained:



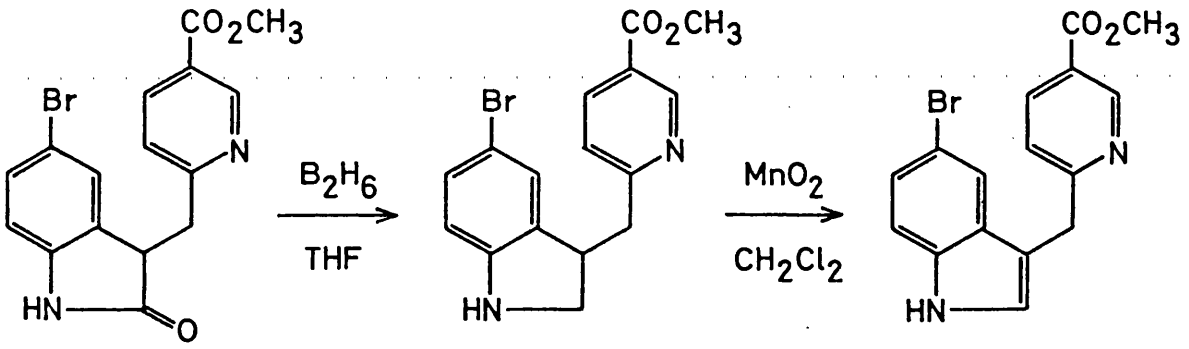
124 125(40%) 126(26%)

The formation of the latter compound is not without precedent: Similar ring cleavage, in which the oxindole 3-substituent bears a hetero atom positioned a suitable distance from the oxindole carbonyl group, is not unknown[71]. Formation of the indole is brought about by a rearrangement which may be rationalised in the following manner:



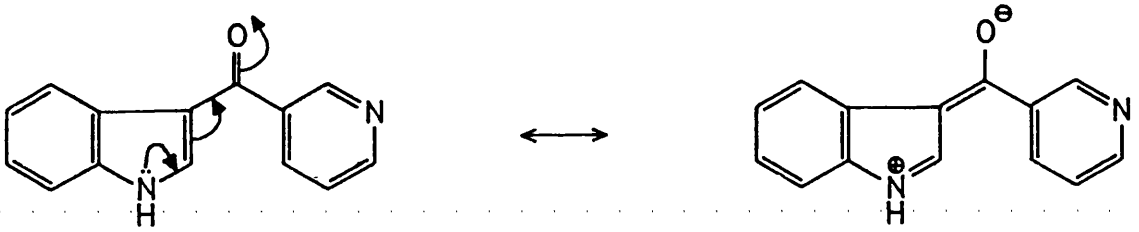
126

In France, a group of workers preparing precursors of lysergic acid derivatives effected the reduction of oxindoles to indoles using diborane in tetrahydrofuran[72], with subsequent aromatisation by manganese dioxide in dichloromethane solution:



A second attempt at the reduction of the oxindole (118) with lithium aluminium hydride in refluxing tetrahydrofuran returned unchanged starting material even when the reaction was in progress over a period of twenty-four hours. Catalytic hydrogenation of this compound at a pressure of 120 p.s.i. in ethanol solution gave a complex mixture of products which could not easily be identified, and whilst some of the reduction methods referred to here seemed quite attractive, it was decided that the overall route did not constitute a viable synthesis of 3-[1-(3-pyridyl) ethyl]indole (29) and derivatives thereof. Furthermore, isatin derivatives themselves are seldom prepared in yields of better than 50%[73].

By contrast, treatment of indol-3-yl-3-pyridyl methanone (111) with methylenetriphenylphosphorane under Wittig reaction conditions with subsequent reduction seemed more attractive. Initially, however, it was necessary to protect the indole nitrogen atom by substitution with an electron-withdrawing group in order to minimise the mesomerism associated with the amino-enone unit of the parent compound, and to induce greater true ketone-like properties at the carbonyl centre:



As a result of this treatment, changes in the carbonyl stretching frequencies were observed in the infra red spectra of derivatives: The unsubstituted compound has $\nu_{C=O}$ 1590cm^{-1} whereas 1-phenylsulphonylindol-3-yl-3-pyridyl methanone (127) shows a carbonyl frequency shift to 1625cm^{-1}. The effect is slightly less marked in the case of the simple acetylated compound (128), which has $\nu_{C=O}$ 1615cm^{-1}. Regrettably, it did not prove possible to prepare the trifluoracetyl derivative (129) despite a number of attempts under various conditions. It seemed most likely that the protecting group was hydrolysed off under the conditions employed to isolate the product and as such the trifluoracetyl group would not have been suitable for its intended purpose.

On subsequent reaction, the acetyl derivative (128) was found to undergo hydrolysis itself, returning only the de-acetylated ketone (111). This was not altogether surprising since the electron-withdrawing effect of the N-acetyl group was not expected to be very great and hence the electrophilic nature of the carbonyl carbon atom was still quite low. On the other hand, the N-phenylsulphonylated compound (127) underwent a smooth reaction with methyltriphenylphosphonium bromide and sodium hydride in anhydrous tetrahydrofuran at reflux to give a brown oil. Owing to the greater

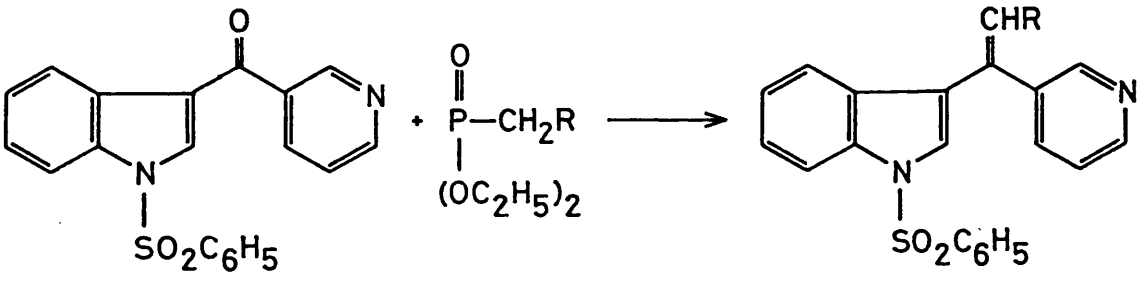
solubility of the phosphorus decompositon products in tetrahydrofuran compared to diethyl ether, it was necessary to chromatograph the crude product on a column of silica gel using chloroform as eluent. The product was then obtained as an orange oil which slowly solidified on standing but defied repeated attempts at recrystallisation. The presence of some starting ketone was also indicated in the crude product from comparisons on TLC. This was to be expected, since the alkaline hydrolysis of N-phenylsulphonylated indoles is well documented[74,75]. Despite considerable efforts to purify the product, including further column chromatography on alumina, there was strong evidence to suggest that it was still contaminated with triphenylphosphine oxide. Infuriatingly, it was possible to obtain this compound as a pure crystalline product from a second column fractionation.

In the ^{1}H nuclear magnetic resonance spectrum of 1-(1-phenylsulphonylindol-3-yl)-1-(3-pyridyl)ethene (130), the two methylene protons appeared as a broad singlet at δ5.76. The pyridine C-2 proton appeared as a doublet at δ9.10 and the C-6 proton gave a double doublet at δ8.86. The remaining protons gave a complex signal between δ8.70 and δ7.10 which could be crudely resolved into signals arising from protons within the deshielding zone of the sulphonyl group and proton signals which remained unaffected. More rigorous interpretation of this region of the spectrum was complicated by the superposition of the triphenylphosphine oxide spectrum.

In view of the difficulty experienced in obtaining a pure sample of this compound it was decided to proceed with the subsequent steps and assess the overall viability of the route. On treatment with lithium aluminium hydride followed by hydrolysis, the

phenylsulphonyl group was removed but no other modifications were discernible from the spectral evidence. Hydrogenation was then carried out at 100 p.s.i. in ethanol solution using a platinum oxide catalyst. The reaction mixture was then coarsely filtered through Kieselguhr and washed down a short column of silica gel before removing the solvent to give a gum. This was taken up in chloroform, washed with a solution of brine, and the dried chloroform solution was evaporated to give an oil which yielded 3-[1-(3-pyridyl)ethyl] indole (29) on trituration with diethyl ether. However, the overall yield in relation to the starting ketone (127) was only 24% which meant that this was a less attractive process than the existing indolylmagnesium bromide reaction with 3-(1-chloroethyl)pyridine (73).

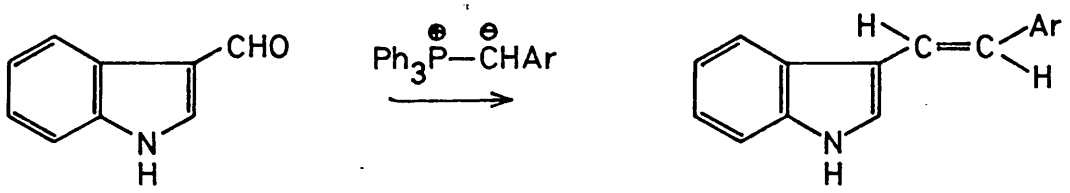
In spite of this, the Wittig reaction may yet provide a useful synthetic route to various 11-substituted - 11-demethyl-ellipticine derivatives <u>via</u> indol-3-yl-3-pyridyl methanone (111). Indeed, work is currently in progress[76] to optimise reaction conditions for treatment of the ketone (127) with ylides derived from triethylphosphonoacetate (131) and diethylphosphonoacetonitrile (132). So far these reactions have yielded 30% and 70% of the respective methylene compounds with none of the associated problems of contamination with phosphorus by-products.



127

131, R = $CO_2C_2H_5$
132, R = C≡N

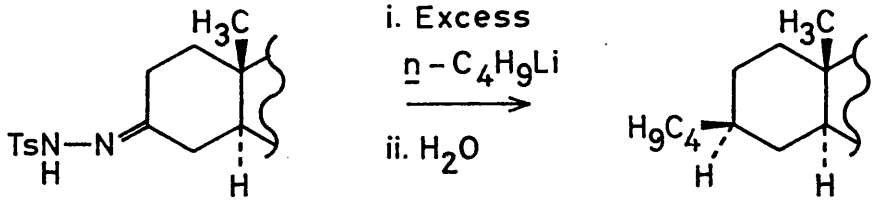
In this modified form the reaction compares well with the work conducted by Tewari and Gupta[77] in which a number of 1-aryl-2-(3-indolyl) ethylenes (134) were prepared by olefination of indole-3-carboxaldehyde (133) with a variety of ylides. Typically:



133 134

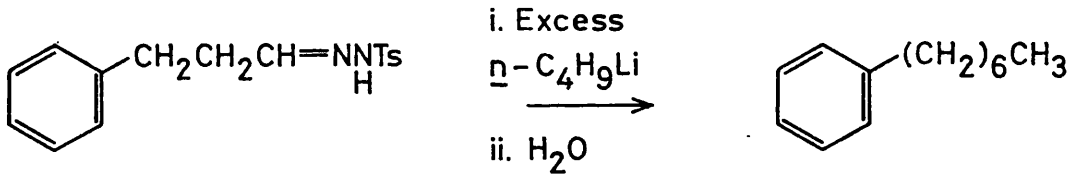
Taylor and Joule[49] have also successfully employed the Wittig reaction in the preparation of ellipticine and analogues (see page 41).

A further interesting possibility for the conversion of indol-3-yl-3-pyridyl methanone (111) into 3-[1-(3-pyridyl)ethyl] indole (29) was the preparation of the corresponding p-toluenesulphonyl hydrazone (135). In certain cases the tosylhydrazone is reported to then undergo alkylation in the presence of an excess of alkyl-lithium, such as in the preparation of 3β-n-butyl-5α-cholestane (137) from 5α -cholestan-3-one tosylhydrazone (136)[78,79].



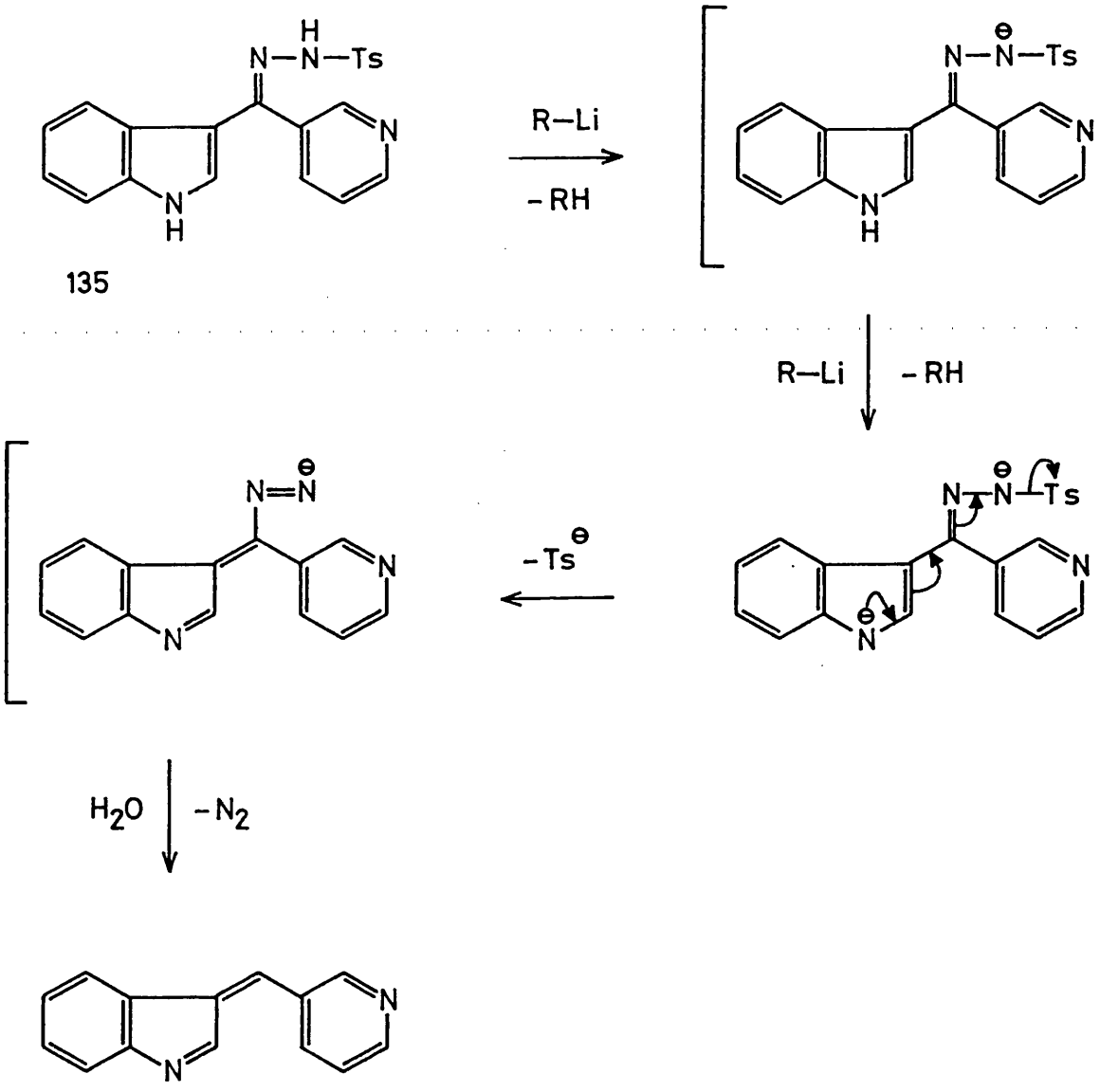
136 137

Most typically this reaction is carried out on aldehyde tosylhydrazones, as described by Vedejs and Stolle[80]. For example, their treatment of 3-phenylpropanal tosylhydrazone (138) with excess n-butyllithium at -78° resulted in a 49% conversion to the saturated hydrocarbon (139):



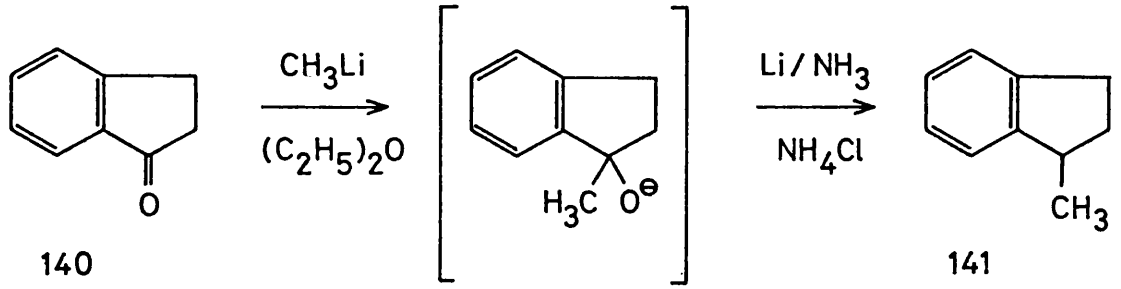
138 139

With ketones this procedure is more generally used to prepare olefins, and in particular to prepare conjugated dienes from α,β -unsaturated ketones which might otherwise be obtained only with extreme difficulty[81-83]. Olefin formation is possible because dianion formation usually predominates in ketones having enolisable α-hydrogen atoms. Whilst indol-3-yl-3-pyridyl methanone (111) does not suffer from this drawback, it is possible to envisage the interfering process which might take place involving deprotonation of the indole nitrogen atom:



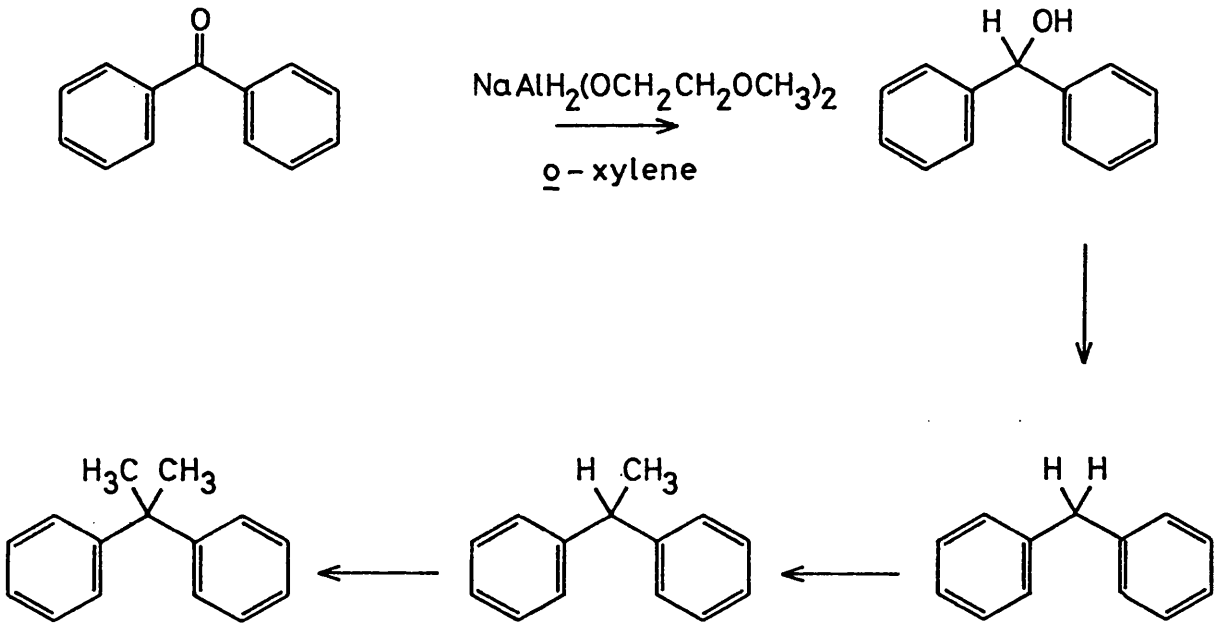
135

The product here is similar in many respects to the intermediate postulated in the formation of 1-(indol-3-yl)-1-(3-pyridyl)ethene (110) from methyllithium treatment of the starting ketone. Hall and Lipsky[84,85] have reported another alkylation-reduction procedure for aromatic ketones and aldehydes in which alkylation was achieved using an organolithium reagent, followed by lithium-ammonia reduction without isolation of intermediates. Using this technique it was possible, for example, to effect the conversion of indan-1-one (140) to 1-methylindane (141) in better than 90% yield:



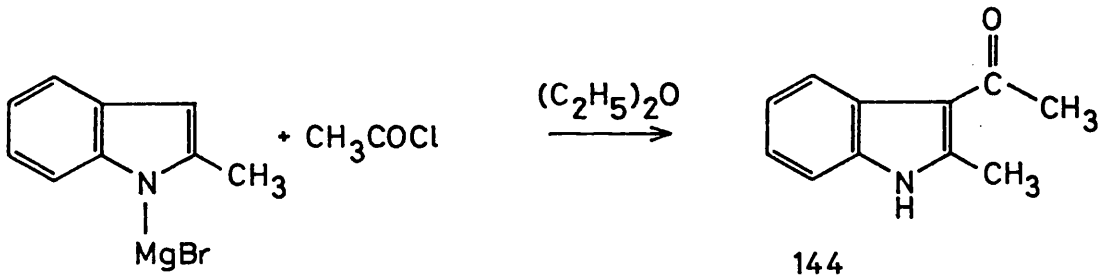
140 141

A rather more elegant procedure has been reported by Cerny and Malek[86] using sodium bis-(2-methoxy)aluminohydride which acts as a dual-purpose reagent, effecting both reduction and alkylation in a single-pot reaction. The difficulty here lies in terminating the reaction at the appropriate time. For instance, in the treatment of benzophenone with this reagent, the intermediately-formed diphenylmethane (142) bears two active hydrogen atoms which undergo selective alkylation so that the final product might be 2,2-diphenylpropane (143) if the reaction is allowed to proceed for too long:



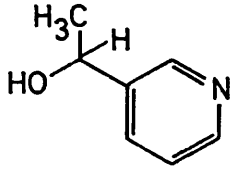
143 142

At the present time it would appear that the best method of preparation of 3-[1-(3-pyridyl)ethyl]indole (29) is the one which has been most commonly used, namely the condensation between indolylmagnesium bromide and 3-(1-chloroethyl)pyridine (73). In essence this reaction has the potential to give much better yields since indolylmagnesium bromide has been successfully employed on many previous occasions. For example, in the preparation of 3-acetyl-2-methylindole (144) from 2-methylindolylmagnesium bromide and acetychloride, Oddo and Sessa[87,88] report a yield in excess of 85% of pure product.



144

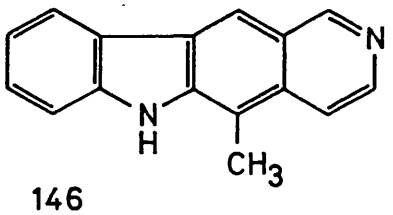
Such a result would seem to indicate that the problem here lies with the pyridine moiety and its inherent instability under the conditions employed for this reaction. In the future it is hoped to overcome this difficulty by employing a suitably-substituted derivative of the alcohol (145). The p-toluenesulphonyl derivative is an obvious possibility.



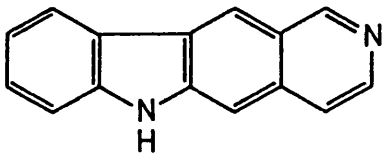
145

Synthesis of 5-substituted-11-methyl-6H-pyrido[4,3-b] carbazole derivatives:

The role of the methyl groups in ellipticine is not yet fully understood, although their importance to antitumour activity became apparent from work conducted by Le Pecq et al.[16] who observed that neither 11-demethylellipticine (146) nor 5,11-didemethylellipticine (147) showed any activity against mouse L1210 leukaemia. This inactivity, shown by a decrease of some three pK_a units compared with ellipticine itself, is accompanied by a corresponding ten-fold decrease in the DNA binding affinity at physiological pH (See Table 1, page 12). Clearly, if these materials are less strongly bound in DNA, they will be less effective as intercalating drugs. However, it should be emphasised that a high DNA binding constant is not a sufficient criterion for high antitumour activity.



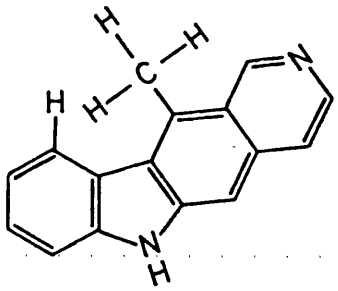
146



147

It is thought that alkyl substituents at positions 5- and 11- of the pyridocarbazole skeleton might enhance the lipophilicity of the molecule, facilitating easier passage through the cell membrane, but the reason that their absence should have such a marked effect on the pK_a value may also be due to steric effects. Pyrido [4,3-b] carbazole (147) is a planar arc-shaped molecule, but its 11-methyl derivatives are forced into non-planar arrangements because of the significant peri-interaction between the substituent and the hydrogen atom at C-10. Thus it seems probable that the geometric

requirements for antineoplastic activity are quite critical.

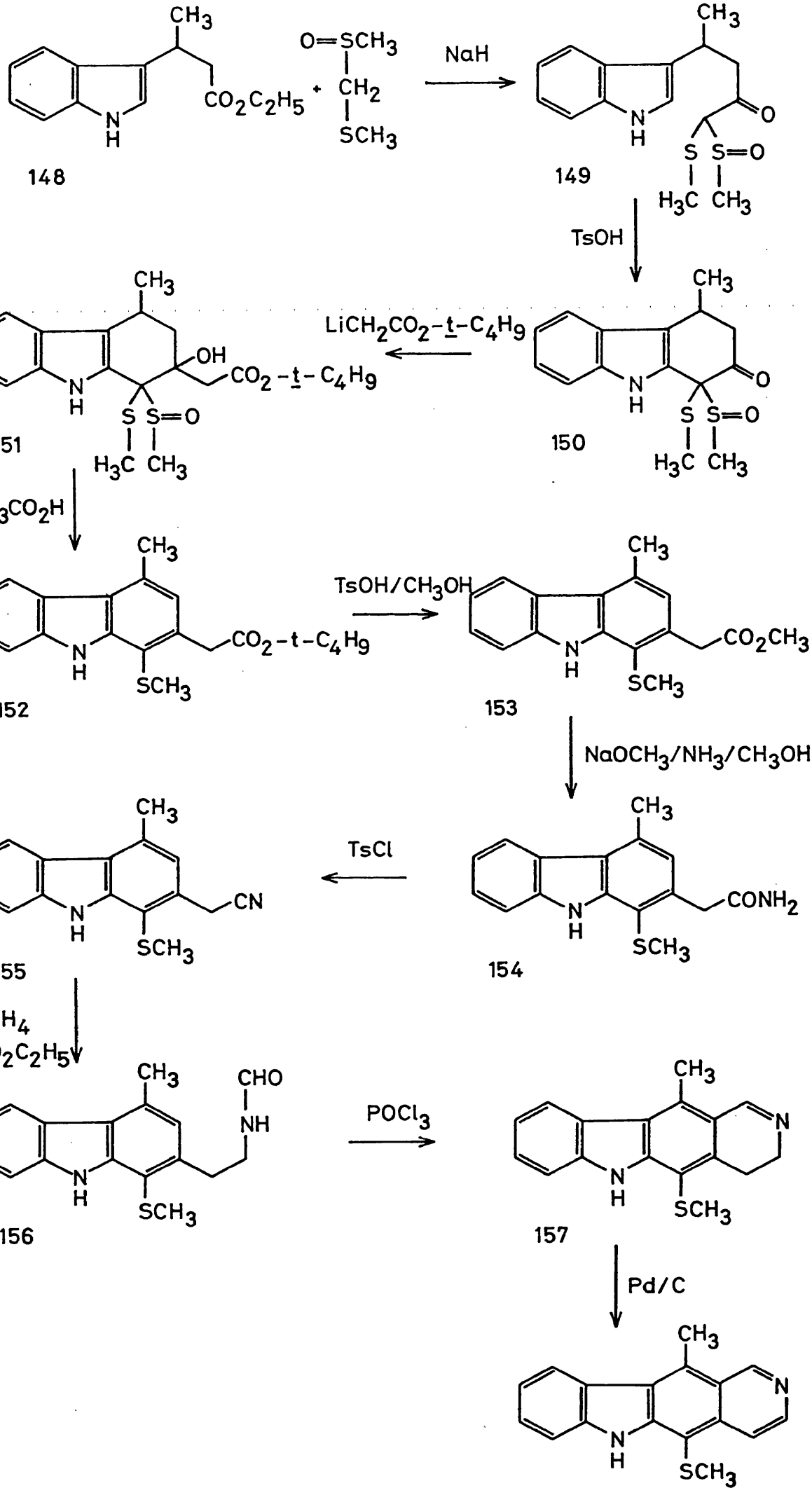


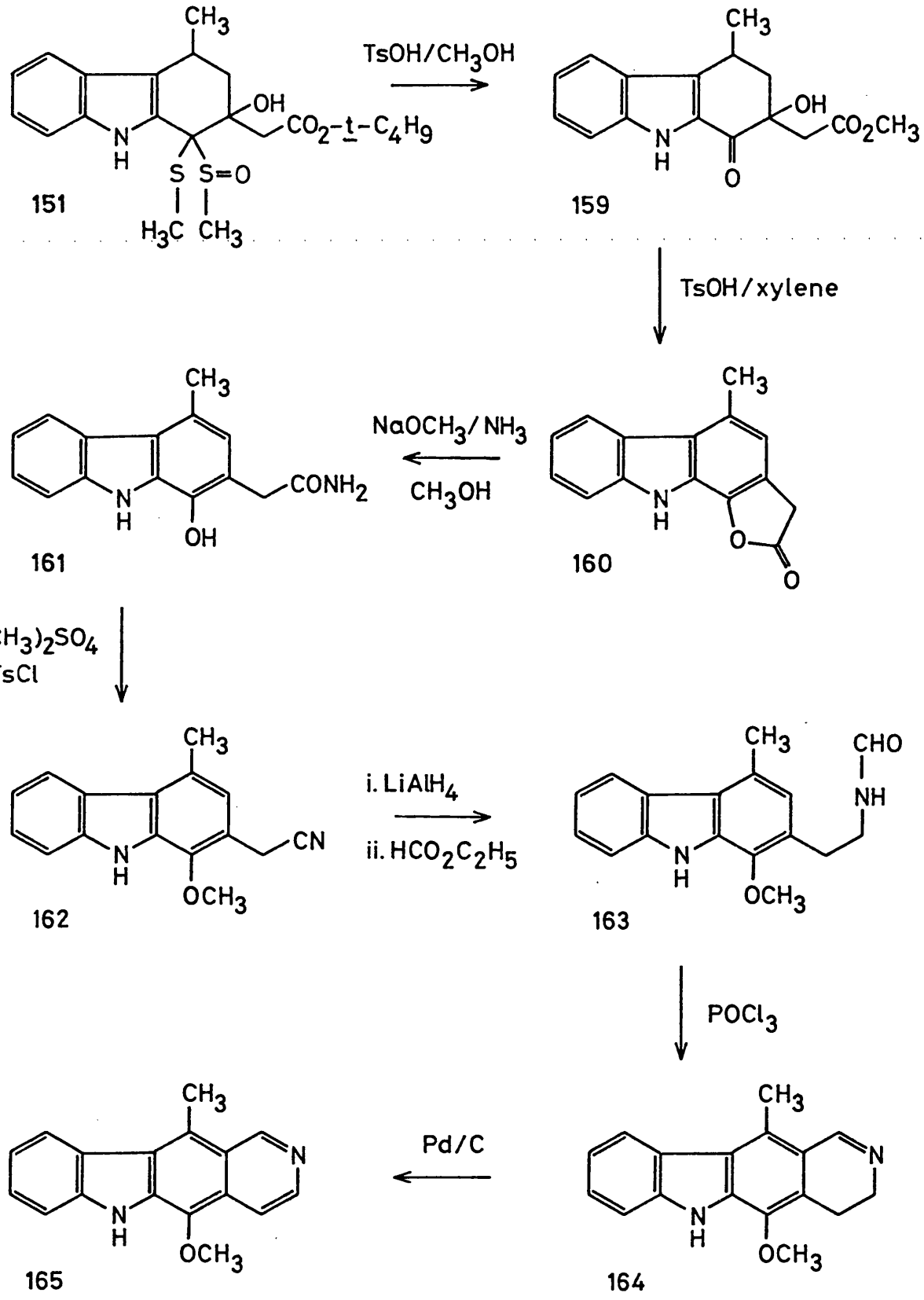
Since the ellipticine synthesis developed in this laboratory lends itself so readily to the preparation of analogues bearing different 5-substituents whilst retaining the 11-methyl group, it was felt that these observations warranted further investigation, particularly the question of lipophilicity which should be further improved if the 5-alkyl side-chain is lengthened.

Recent publications indicate that other chemists are active in this area. For example, a Japanese group[89] have synthesised 5-methylthio- (158) and 5-methoxy- (165) analogues of ellipticine using the indole ethylbutyrate ester (148) as a common starting material. Condensation of this compound with formaldehyde di-methylthioacetal-S-oxide (FAMSO) in the presence of sodium hydride proceeded smoothly to the β-keto sulphoxide (149) which was cyclised in anhydrous p-toluenesulphonic acid at 60° to the ketone (150). This was then treated with tertiary-butyl lithio-acetate to give the ester (151). In the preparation of the methyl-thio compound the ester was aromatised with acetic acid in refluxing xylene to give (152) which was trans-esterified with p-toluenesulphonic acid in methanol to the methyl ester (153). This was treated with methanolic ammonia containing sodium methoxide to give the amide (154) which was dehydrated with p-toluenesulphonyl chloride to the nitrile (155) and then reduced with lithium

aluminium hydride. The resultant amine was heated with ethyl formate to give the formate (156). Finally, cyclisation was effected with phosphorus oxychloride in refluxing toluene to the dihydroellipticine analogue (157) which was most effectively dehydrogenated using palladium on charcoal in refluxing decalin to give the aromatic tetracycle (158), as shown on the following page.

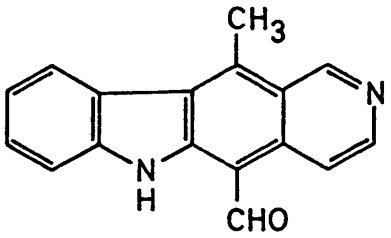
In the synthesis of the methoxy analogue, the tertiary-butyl ester (151) was treated with a small amount of p-toluenesulphonic acid in methanol which resulted in cleavage of the dithioacetal group and trans-esterification to the ketone (159). Acid-catalysed dehydrative aromatisation of this material was achieved by refluxing in xylene in the presence of p-toluenesulphonic acid to give the lactone (160) which was cleaved to the amide (161) using methanolic ammonia and sodium methoxide. The phenolic group was methylated with dimethyl sulphate and the resulting methoxy compound was dehydrated to the nitrile (162) in the same manner as described above. Reduction and formylation were also carried out as before, and the formate (163) was cyclised with phosphorus oxychloride to give the tetracycle (164). This could be aromatised directly to the methoxy compound (165) with palladium on charcoal, but in fact it was found that better results were achieved by first reducing the material to the tetrahydro derivative with sodium borohydride and then carrying out the dehydrogenation step in the usual manner: See page 74.


148
NaH
149
TsOH
150
LiCH2CO2-t-C4H9
151
CH3CO2H
152
TsOH/CH3OH
153
NaOCH3/NH3/CH3OH
154
TsCl
155
i. LiAlH4
ii. HCO2C2H5
156
POCl3
157
Pd/C
158

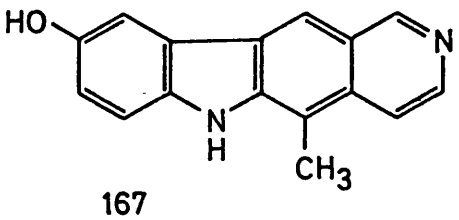

TsOH/CH3OH
TsOH/xylene
NaOCH3/NH3
CH3OH
i.(CH3)2SO4
ii. TsCl
i. LiAlH4
ii. HCO2C2H5
POCl3
Pd/C
151
159
160
161
162
163
164
165

These workers state that in the near future demethylated 5-mercapto- and 5-hydroxy-analogues will be available. At the moment no biological activity studies on the first two products have been reported.

Also of interest here is the discovery of a naturally-occurring 5-substituted ellipticine derivative, the so-called 17-oxoellipticine (166) isolated from the bark of Strychnos dinklagei Gilg. by Michel, Tillequin and Koch[90]. Again, the antitumour activity of this new alkaloid has not yet been assessed.



166

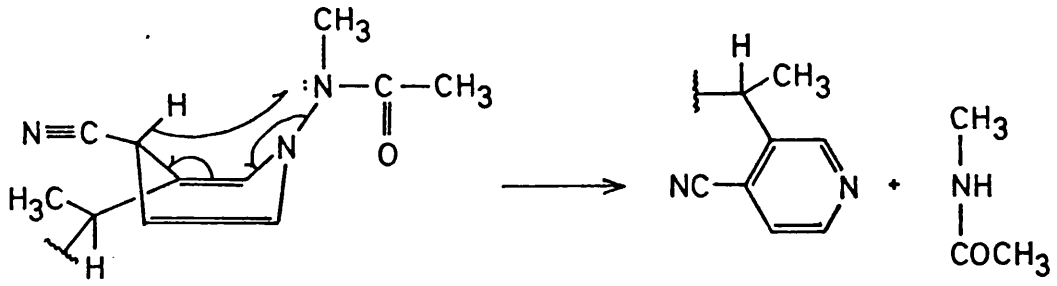


167

9-Hydroxy-11-demethylellipticine (167) has recently been synthesised using a variety of known methods by Gouyette et al.[91], and its activity compared with the corresponding ellipticine derivative. In general it was observed that derivatives lacking the 11-methyl group were less toxic than the parent ellipticines and that demethyl compounds were less effective in untwisting the DNA helix, having a correspondingly weak DNA binding affinity. Although the authors acknowledge that the number of demethyl-ellipticines tested was too small to establish a firm relationship between DNA binding affinity and antitumour activity, their results are consistent with previous observations.

Although the condensation between 3-(1-chloroethyl)pyridine (73) and indolylmagnesium bromide was found to be the best method of preparation of 3-[1-(3-pyridyl)ethyl] indole (29), this procedure did not lend itself to large scale preparations. Any attempt to scale up the reaction in order to give a greater weight of product than approximately 3g resulted in an inexplicable diminution in yield. But by using a batch - preparation technique, sufficient quantity was obtained for conversion into 1-acetyl-3-{1-[3-(4-cyano)-pyridyl] ethyl} indole (78b) using a reaction sequence developed in this laboratory and which has been described elsewhere (see Scheme 20, page 37).

Conversion of the methiodide compound (77) into the required cyanopyridine was made more effective by dissolving the salt in a large volume of hot water containing a little ethanol. On cooling, a finely-divided suspension was obtained which was then treated with aqueous potassium cyanide in the presence of ammonium chloride to give the dihydropyridine precursor as a gum. This was taken up in chloroform and thoroughly washed with water. The solvent was then removed and the product was obtained as an orange oil which could be converted to the fully aromatic pyridine (78) either by warming an ethanolic solution of the compound or upon irradiation with ultra violet light from a medium pressure source. The photochemical decomposition is probably a concerted process in which the dihydropyridine adopts a boat conformation to eliminate N-methylacetamide as shown on the following page:



However, this point is not certain and neither is the mechanism of the apparent thermal decomposition. Whatever the true order of events, this is an efficient reaction and usually at this stage the pyridylnitrile was chromatographed on a column of basic alumina to hydrolyse the indole N-acetyl function and to separate N-methyl-acetamide which is a co-product of the reaction. However, experience has shown that the product isolated from the column fractions does not crystallise and since it is a relatively "heavy" molecule, this led to speculation about the integrity of the nitrile. This compound bears a formal chiral centre, and it was thought that restricted rotation about the bridging ethyl group might lead to diastereomeric forms. Although this may have been a contributing factor, it was found that column separation on silica gel afforded the N-acetyl compound (78b) in crystalline form and thus it seems likely that passage of the original mixture through basic alumina simply resulted in partial de-acetylation, and the resulting mixture of substituted and unsubstituted indoles failed to crystallise.

The inclusion of the N-acetyl group was not considered to be detrimental in succeeding reaction steps since it was felt that the indole-1-position was certain to be de-acetylated by strong base at a later stage.

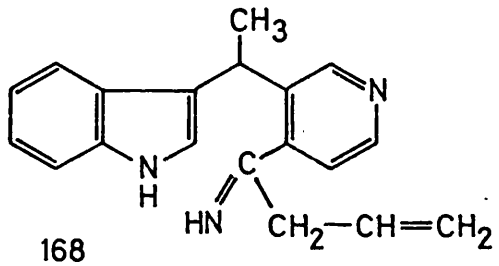
In the attempted preparation of 5-allyl-5-demethylellipticine (169), the cyanide compound (78b) was added to a freshly prepared solution/allylmagnesium bromide and the product was hydrolysed with the intention of obtaining the desired tetracycle in a single-pot reaction. However, only a small amount of material was isolated, as a brown gum which was submitted for mass-spectral analysis. Two molecular ions were detected at m/e 289 and m/e 314 and these were speculatively assigned to unchanged starting material and to 6-acetyl-5-allyl-5-demethylellipticine, respectively. In many respects this result is surprising because previous work has tended to suggest that Grignard reagents are insufficiently reactive to attack this hindered nitrile[92]. In addition, retention of the indole N-acetyl group was totally unexpected and raises doubts about the ease with which this function undergoes hydrolysis.

When the reaction was repeated on a larger scale, a solid material was isolated from the reaction mixture. Infra-red analysis of this compound showed it to be unreacted starting material and in the mass spectrum there was no evidence that any ring-closed product had been formed.

The second approach to this preparation involved the use of allyllithium as an alternative nucleophile. This was obtained as a solution in anhydrous tetrahydrofuran by cleavage of allyl-phenyl ether with lithium metal[93], and using the titration technique of Watson and Eastham[94] it was estimated that this procedure gave a 45% conversion of the ether to allyllithium.

A solution of the cyanide compound (78b)in dry tetrahydrofuran was added cautiously to the organometallic reagent at reduced temperature, and after extraction the product was obtained as a brown oil. A small portion of this material was retained for

infra-red analysis which showed a band at 1660cm^{-1} corresponding to the >C=NH stretch and providing further evidence to support the structure of the presumed intermediate imine (168). The -C≡N stretching band and the carbonyl absorption of the indole N-acetyl group were absent from this spectrum.



168

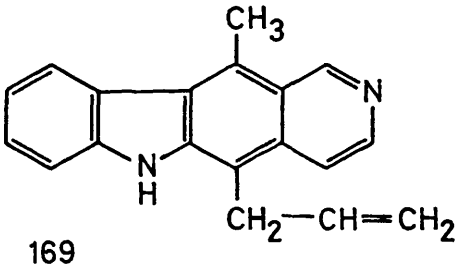
The mass spectrum showed a parent molecular ion peak at m/e 289, and whilst this value also corresponds to the starting nitrile (78b), there were no other common spectral features. Previous experience with these compounds has shown that they are not readily isolable in pure form so full characterisation was not carried out.

The crude oil was treated with dilute acetic acid on a steam bath for about an hour and, following neutralisation with sodium hydrogen carbonate, the mixture was extracted with chloroform. The combined extracts were dried and reduced in bulk to give the product as a viscous brown oil. On dissolving in a little chloroform and triturating with ether, this oil afforded a green solid which defied subsequent attempts of recrystallisation. Analysis of this material by TLC showed it to be impure but the bulk of the material fluoresced as a bright yellow spot which moved only slightly in solvents of medium polarity.

Column chromatography was employed to improve the purity of this material, but with only limited success. The eluted substance

could still not be induced to crystallise and was obtained as an orange-coloured amorphous solid.

Owing to the nature of this material, full spectral characterisation could not be achieved, but in its mass spectrum the parent molecular ion was obtained at m/e 272 with fragment peaks at m/e 257 and m/e 245 corresponding to loss of $-CH_3$ and $-CH=CH_2$ respectively. These observations tend to indicate that the desired tetracycle (169) has been formed, a supposition which is confirmed by the ultra violet spectrum. This includes absorption maxima at 278, 292 and 298 nm so characteristic of ellipticine derivatives.



169

Satisfactory ^1H nuclear magnetic resonance data could not be obtained from this material in its impure state so it was not possible to determine whether the allylic double bond was in conjugation with the aromatic nucleus or as shown in the figure above, or indeed whether both isomers occurred together. Reduction of the olefinic double bond was strongly considered in order that the compound might be compared with the 5-propyl analogue which could be easily synthesised directly. This possibility was rejected, however, since the immediate interest was in the allyl structure itself, which it was felt may have exhibited unusual biological activity: Vinyl groups are commonly converted to epoxy functions *in vivo*[95], and these may then be further

metabolised to diols or alcohols. Thus it might be possible to form a more soluble derivative of ellipticine, enabling smaller doses to be administered for an equivalent antitumour effect.

When the reaction between the nitrile (78b) and allyllithium was repeated at -78°, the same oily intermediate was obtained, and on acid hydrolysis this gave a product which was similar in every respect to that obtained from the first preparation. In this case, also, separation of the ellipticine derivative in pure form could not be achieved.

In a slightly more ambitious reaction, attempts were made to emulate the work of Bisagni et al.[47,48] by introducing the 3,3-diethylaminopropylamino group at the 5-position of the pyrido-carbazole system. The immediate problem here was lithiation of 3,3-diethylaminopropylamine, since an initial reaction between the amine and freshly-cut lithium pieces in boiling benzene did not appear to work, although a slight red colouration was clearly discernible after a couple of hours. However, when a mixture of hexamethylphosphoric triamide (HMPT), benzene and the amine were cooled to -78° and treated with n-butyllithium solution in hexane an orange-coloured solution of the lithiated amine was obtained. The role of the HMPT here is two-fold in that it enables metalation to take place and it also enhances the nucleophilicity of the product for subsequent reaction.

A solution of the nitrile (78b) was added dropwise to a stirred solution of the lithiated amine at reduced temperature resulting in a deep red-coloured mixture which was allowed to warm up to room temperature overnight. After work-up, the product was extracted into chloroform and when the solvent mixture was removed under reduced pressure, a brown oil was obtained, a small portion

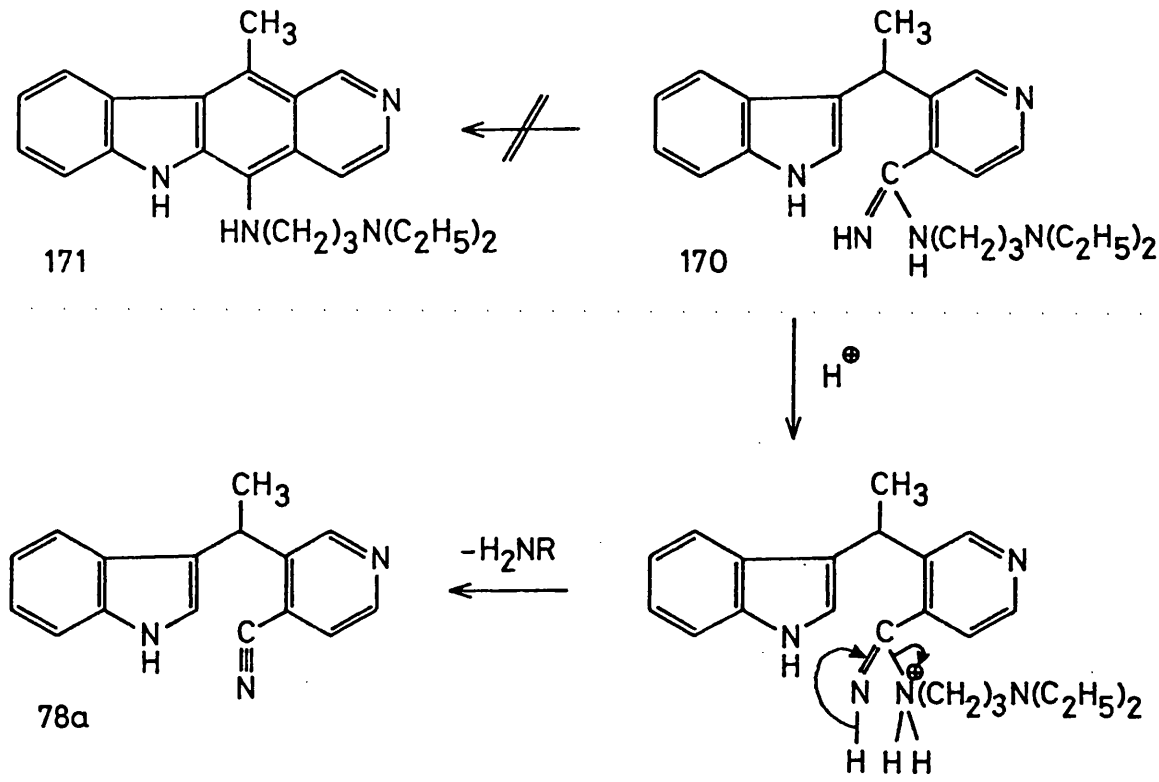
of which was submitted for mass-spectral analysis. This was thought to be a solution of the desired imine (170) in HMPT, a speculation which was confirmed by the presence of the parent molecular ion peak at m/e 377 in the mass spectrum. This also showed that there was some unreacted starting material present, indicated by peaks at m/e 289 and m/e 247.

Subsequently, when this material was treated with dilute acetic acid on a steam bath for an hour, no trace of the expected tetracycle (171) could be found. Once again the presence of HMPT made isolation of the product rather difficult, but following chromatographic separation on silica gel an amorphous brown solid was obtained. The mass spectrum of this compound suggested that it was the de-acetylated nitrile (78a), having a parent molecular ion peak at m/e 247. This structural assignment was confirmed by bands at 3270 and 2220cm^{-1} in the infra-red spectrum, corresponding to the indole N-H and nitrile C≡N stretching frequencies, respectively.

It would seem, then, that the conditions usually employed to effect cyclisation result in the cleavage of the C-NHR bond so that the desired group is lost from the pyridine side chain by acid hydrolysis (see following page).

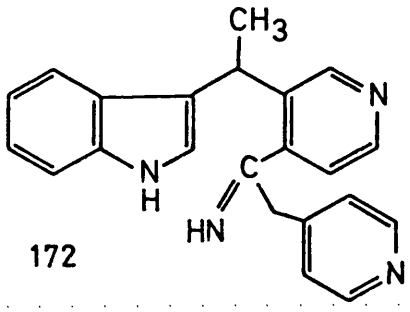
Preliminary attempts at alkaline hydrolysis were discouraging since harsh conditions were required which caused blackening of the substrate in aqueous alcohol solution.

Following these discoveries, work was suspended on the 3,3-diethylaminopropylamino derivative in favour of a much simpler side chain derived from 4-picoline. Both 2- and 4- picolyllithium are readily prepared in good yield from the appropriate methylpyridine and n-butyllithium in tetrahydrofuran solution[96]. The presence of tetrahydrofuran is essential because it enhances the metalating



capability of the n-butyllithium and maintains a greater concentration of the organometallic species in solution. Furthermore, it is also an excellent solvent for the nitrile (78b).

The 4-picolyllithium prepared in this fashion was obtained as a red solution, the concentration of which was determined by titration[94]. A solution of the nitrile (78b) in dry tetrahydrofuran was added dropwise at low temperature and the reaction mixture was then allowed to warm slowly with stirring. Excess organometallic reagent was decomposed by the addition of iced water and the mixture was extracted with chloroform. Evaporation of the solvent under reduced pressure gave an orange oil which smelled strongly of 4-picoline. Nevertheless, the mass spectrum of this substance included a strong molecular ion peak at m/e 340, corresponding to the intermediate imine (172):



172

Confirmation of this structure was obtained from the infra-red spectrum which no longer exhibited the cyanide band at 2220 cm^{-1} or the carbonyl band at 1700 cm^{-1} from the parent nitrile (78b).

The bulk of this material was dissolved in dilute acetic acid and warmed on a steam bath for about one hour. On subsequent basification and extraction the product was obtained as a brown oil which yielded a solid on trituration with diethyl ether. However, analysis by TLC showed this to be a mixture of several components and repeated attempts at recrystallisation from a variety of solvents failed to improve its purity. Column chromatography proved equally unfruitful.

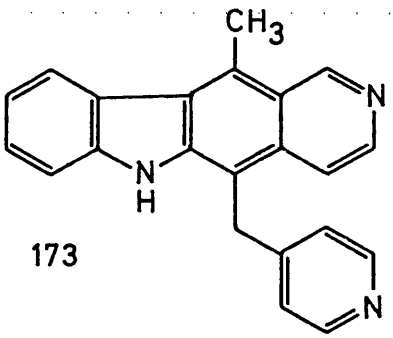
Despite the fact that satisfactory ^1H nuclear magnetic resonance data could not be obtained for this material, there were encouraging signs in the ultra-violet and mass spectra. Although somewhat less intense than the bands in the spectrum of ellipticine itself, the ultra-violet spectrum showed characteristic peaks at λ_{max} 280, 292 and 297 nm. In addition, the mass spectrum included a parent molecular ion at m/e 323, with a fragment peak at m/e 245. It was therefore felt that sufficient evidence existed to suggest that 5-demethyl-5-(4-picolyl) ellipticine (173) had indeed been formed and that a second attempt should be made to prepare it in pure form.

When the experiment was repeated, 4-picolyllithium was obtained as a yellow slurry rather than the red solution which resulted from the initial preparation. In this form the organometallic agent more closely resembled the 4-picolyllithium described by Beumel, Smith and Rybalka[96]. As before, a solution of the nitrile (78b) in anhydrous tetrahydrofuran was added slowly at low temperature, but this time the mixture was gradually warmed to 40° after the completion of the addition to ensure complete reaction. After cautious decomposition with iced water the mixture was extracted into chloroform and evaporated under reduced pressure to give an orange oil which was immediately treated with dilute acetic acid. Work-up afforded a bright yellow solution in chloroform which fluoresced strongly under ultra-violet light. Evaporation of the solvent to low bulk gave the product as yellow needles which were recrystallised from methanol.

Analysis by TLC showed that the material was pure, enabling full spectroscopic characterisation to be carried out. However, the molecular ion peak detected at m/e 288 in the mass spectrum indicated that the reaction had not proceeded as expected. The ^1H nuclear magnetic resonance spectrum included signals characteristic of a n-butyl group: a two-proton triplet was observed at δ3.31 (J=7HZ); a four-proton multiplet at δ2.0 -1.6 due to the intermediary methylene groups and a three-proton triplet at δ1.05 (J=7HZ) due to the terminal methyl group.

These observations implied that the isolated material was in fact 5-n-butyl-5-demethylellipticine (174), a known compound, and that far from being an improved preparation of 4-picolyllithium, the second experiment had produced little or no 4-picolyllithium at all. The formation of the n-butyl derivative (174) was later

confirmed by spectroscopic means, including ^{13}C nuclear magnetic resonance for example, which showed a total of twenty carbon resonances, rather than twenty-two as expected from the 4-picolyl derivative.



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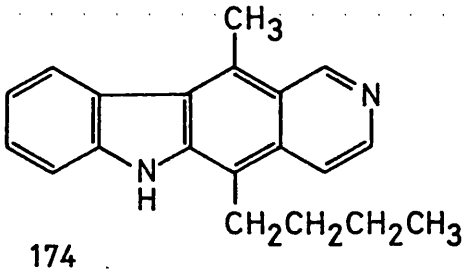


174

In a final attempt to purify the product from the first reaction, the crude material was applied to a preparative silica plate and chromatographed in a solvent mixture of ethyl acetate and petroleum ether. After removal and Soxhlet extraction of the appropriate band and evaporation of the solvent, a small quantity of a pale yellow compound was obtained as fine needles. Mass spectral analysis of this material confirmed that it was 5-demethyl-5-(4-picolyl) ellipticine (173), having a molecular ion peak detectable at m/e 323 and the expected major fragment peak at m/e 245. The ultra-violet spectrum was also much improved compared to that of the crude reaction product.

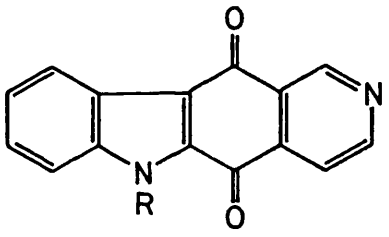
Although only 4mg of this compound had been obtained in pure form, it was possible to acquire its proton magnetic resonance spectrum using Fourier Transform techniques. Understandably, the spectrum was not well resolved and included a very large peak between $\delta 3.5$ and $\delta 3.0$ ppm due to water in the deuterated solvent. This probably masked the resonance of the methyl group attached

at the 11-position. However, the trace showed a singlet absorption at δ11.68 due to the indolic proton and a singlet at δ9.76 due to the proton at C-1. The remaining aromatic protons appeared as a complex pattern between δ8.6 and δ7.0 ppm which could not be resolved without obtaining a better sample. Finally, there was a singlet at δ4.73 which corresponded to the bridging methylene protons.

The conclusion drawn from these results is that more efficient preparative methods should be sought for the lithium alkyl species employed in this synthesis. Without doubt they should be purified prior to use on a precious substrate, and it would probably be valuable to carry out pilot reactions on a readily available analogous compound, such as 3-cyanopyridine, since it is apparent that the titration technique used gives no indication which lithium alkyl species are present.

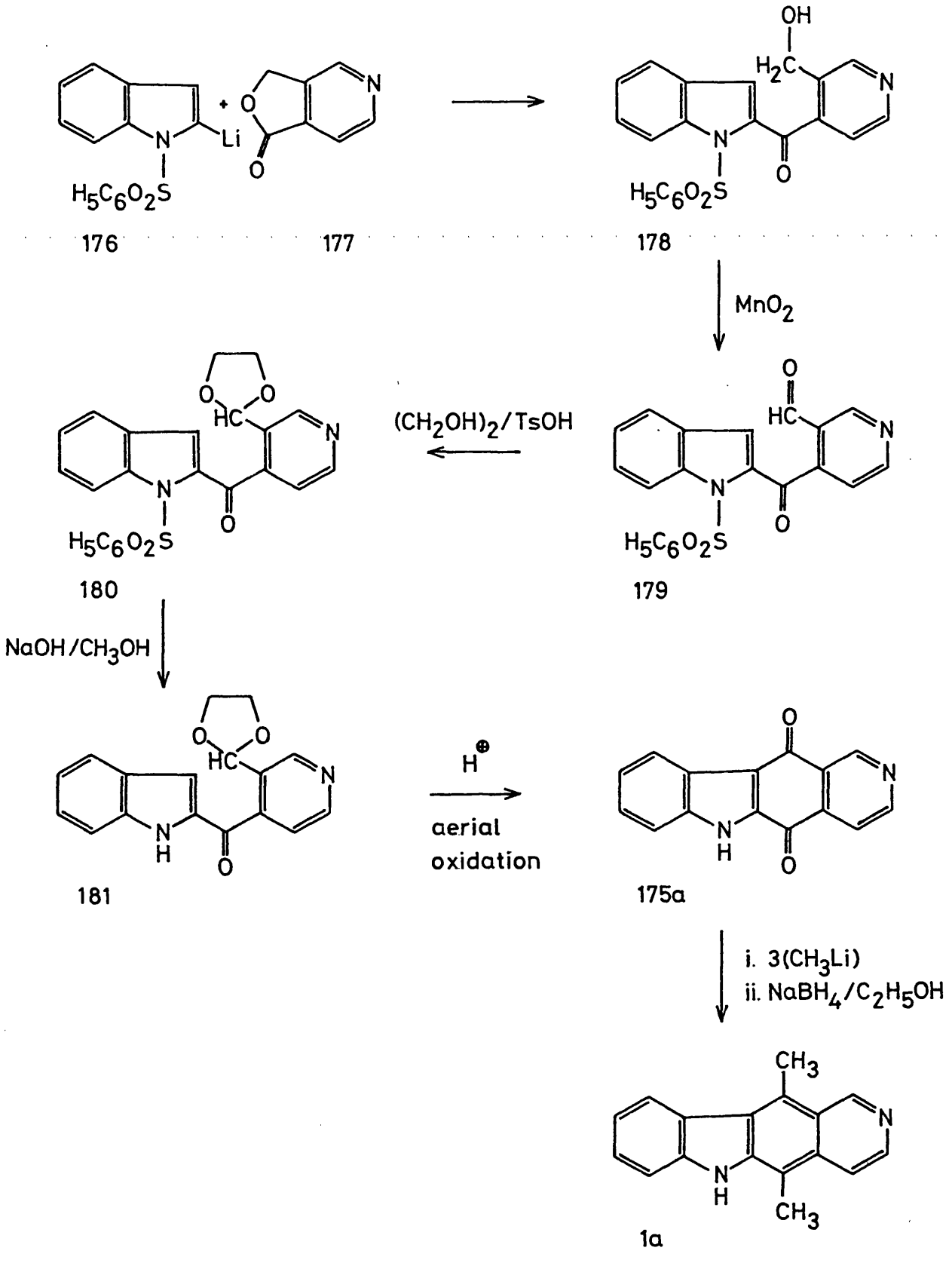
Attempts at an ellipticine synthesis from indol-3-yl-3-pyridyl-methanone (111):

When it became apparent that an efficient synthesis of 3-[1-(3-pyridyl)ethyl] indole (29) could not yet be achieved it was decided to use the carbonyl-bridged analogue as a possible starting point. The stimulus for this work came from two independent syntheses of ellipticine which involved a common precursor, namely the quinone (175):

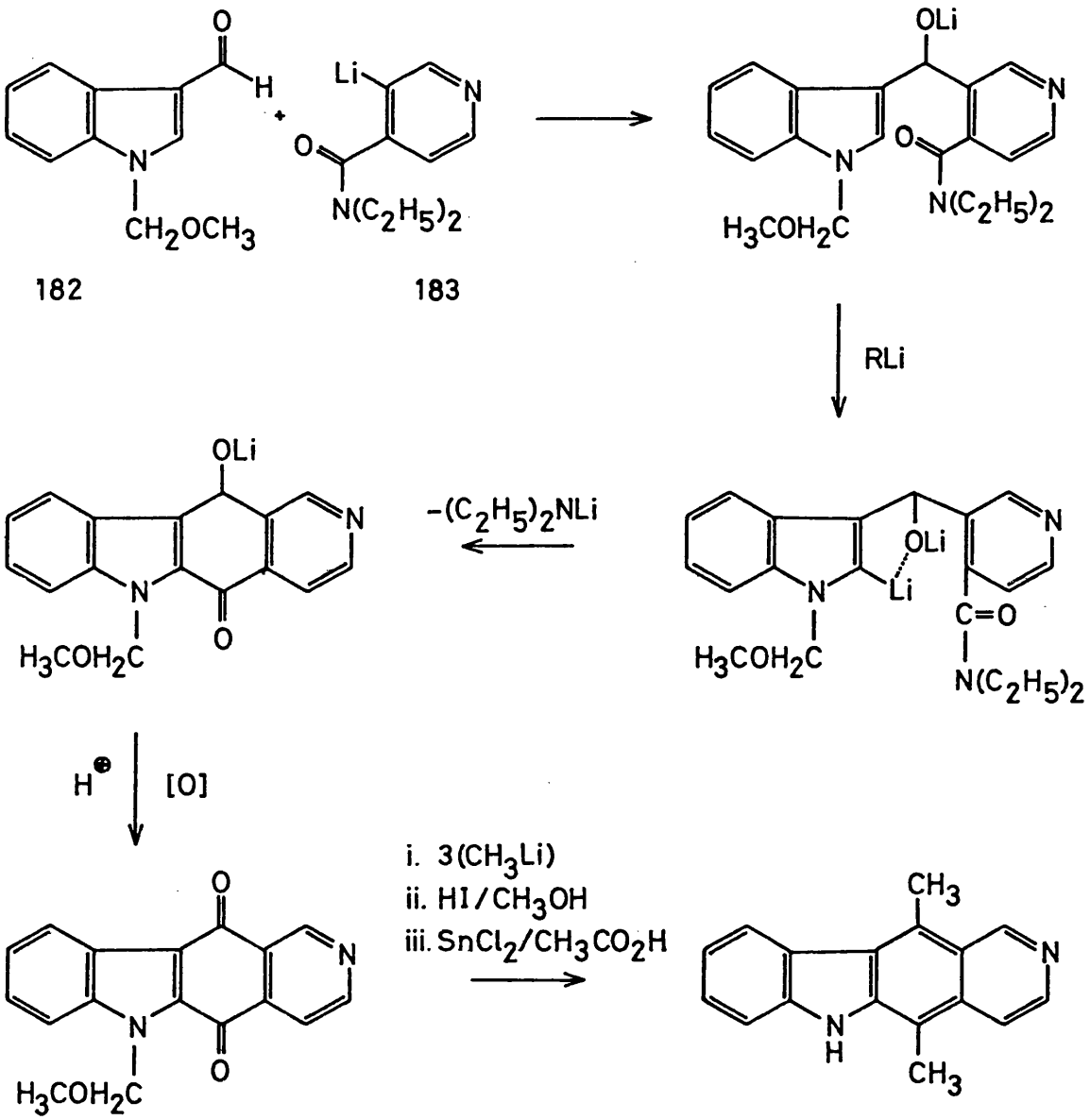


175a, R=H
175b, R=CH_2OCH_3

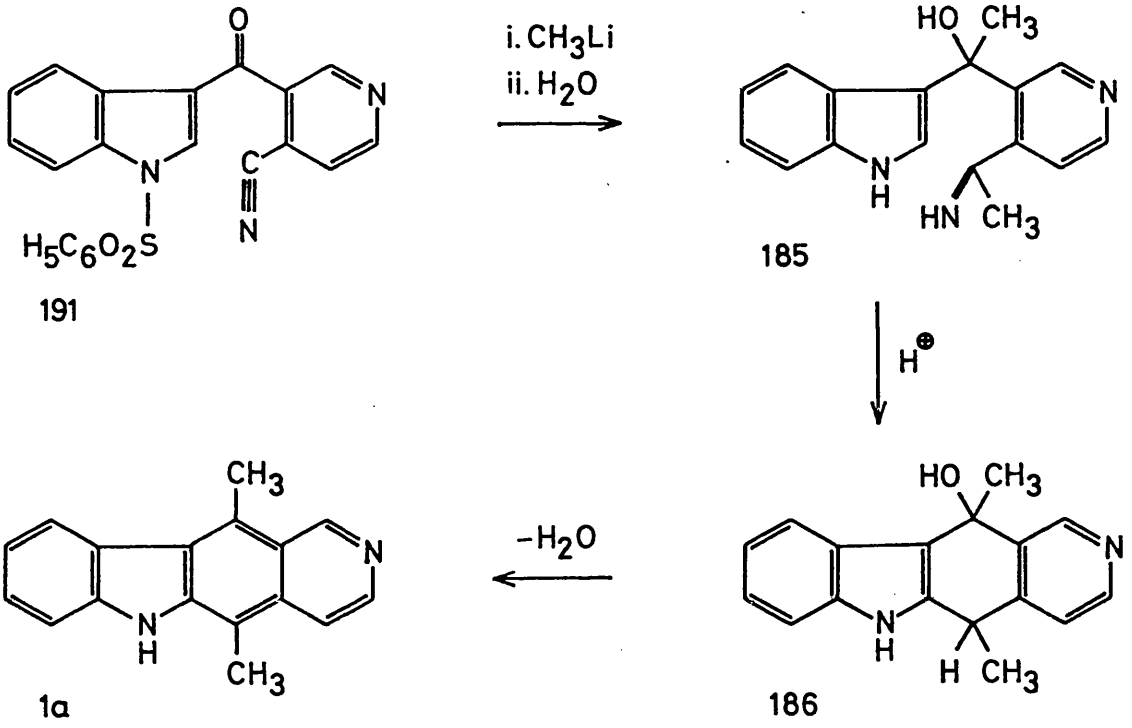
In Great Britain this work was conducted by Joule et al.[97] who reacted 2-lithio-1-phenylsulphonyl-indole (176) with 3-hydroxymethylisonicotinic acid lactone (177) to give the pyridyl alcohol (178). Oxidation with manganese dioxide gave the corresponding aldehyde (179) which was converted into the acetal (180) and then N-deprotected using aqueous sodium hydroxide in methanol. Dilute hydrochloric acid was used for hydrolysis of the acetal (181) and cyclisation to the quinone (175a) proceeded in the presence of air. Treatment of this compound with three mole-equivalents of methyllithium, followed by reduction with sodium borohydride in refluxing ethanol, gave ellipticine in 90% yield from the quinone.


OH
H2C
N
O
Li
H5C6O2S
176
177
178
MnO2
HC
(CH2OH)2/TsOH
180
179
NaOH/CH3OH
H⊕
aerial
oxidation
181
175a
i. 3(CH3Li)
ii. NaBH4/C2H5OH
CH3
1a

A one-step construction of the ellipticine skeleton was carried out in Canada by Watanabe and Snieckus[98], by utilising the reaction of N-substituted indole-3-aldehyde (182) with lithiated N,N-diethyl-isonicotinamide (183). Lithiation of the pyridine moiety was achieved using one moelcular equivalent of secondary butyllithium in the presence of one molecular equivalent of tetramethylethylenediamine (TMEDA) in diethyl ether at -78°. Subsequent treatment of the N-methylenemethoxyquinone (175b) with excess methyllithium, followed by 47% methanolic hydrogen iodide and stannous chloride in a mixture of acetic acid, hydrochloric acid and tetrahydrofuran, gave ellipticine in an overall yield of 40% without the need to isolate any intermediates.



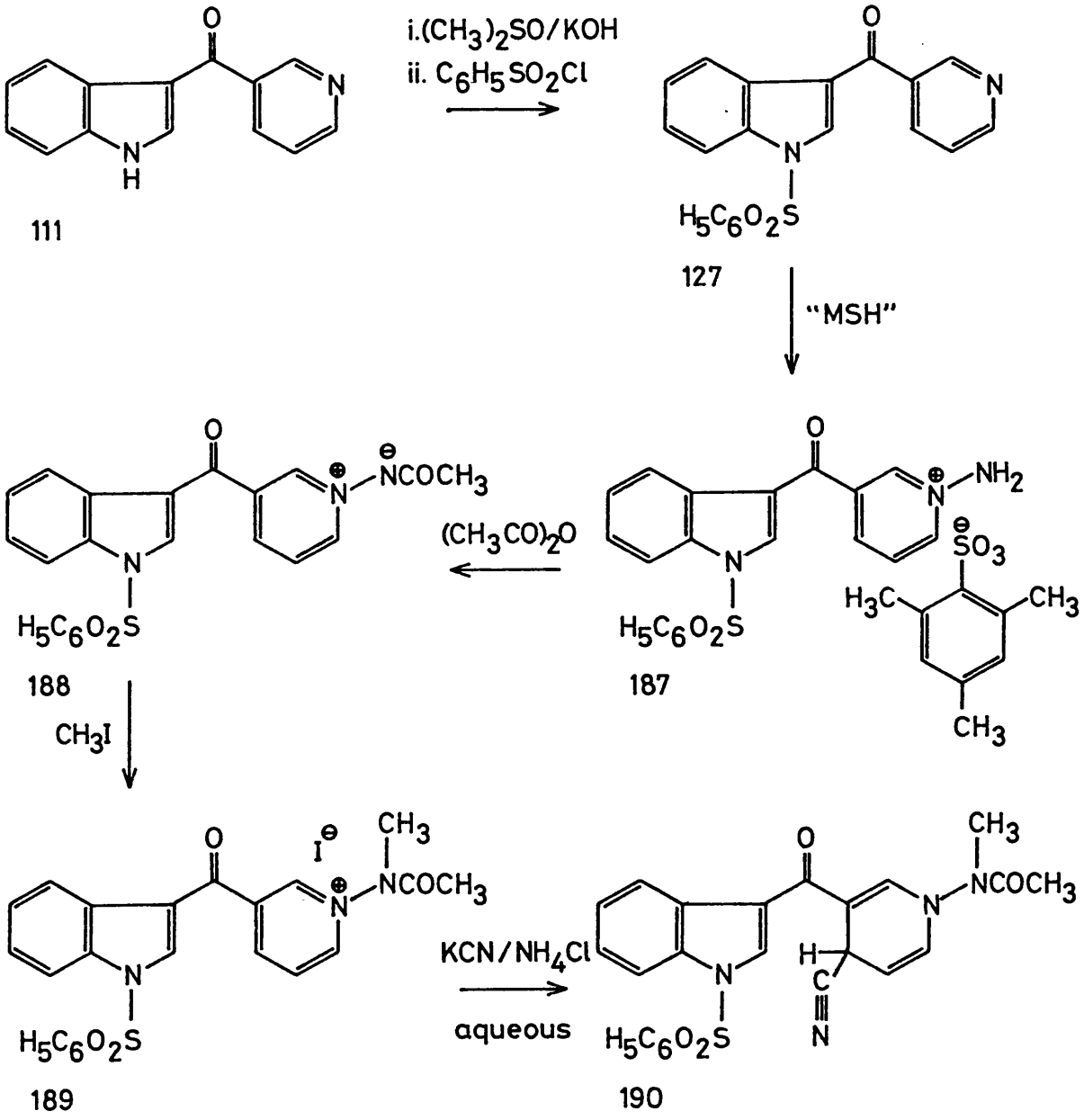
In view of these successes it was therefore intended to mimic the synthesis of the quinone by preparing 1-phenylsulphonylindol-3-yl-3-(4-cyano) pyridyl methanone (191) employing the same method used in the formation of 1-acetyl-3-{1-[3-(4-cyano)pyridyl]ethyl} indole (78b). It was expected that treatment of the nitrile with methyl-lithium would then afford the imine (185) which on subsequent hydrolysis should give either the ring-closed product (186) or the fully aromatic pyrido [4,3-b] carbazole:



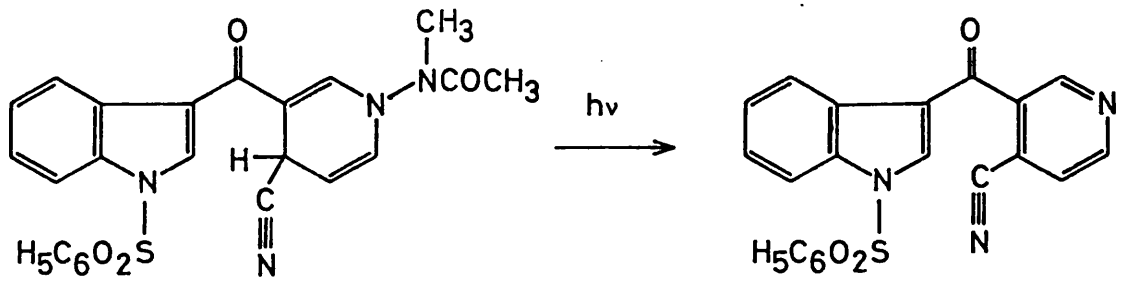
Accordingly, the starting ketone (111) was first reacted with phenylsulphonyl chloride in order to protect the indole nitrogen atom. The reaction was carried out using potassium hydroxide in dimethyl sulphoxide and whilst this gave the N-substituted compound (127) in a fairly pure form, the yield of the reaction was not exceptional. A better method has been reported[97] using a combination of dimethylformamide and sodium hydride.

The ketone (127) in dichloromethane was treated with mesitylene-sulphonyl hydroxylamine (258), and the resulting aminopyridinium

salt (187) was acetylated and quaternised to give the methiodide (189), without isolation of the intermediate product (188). On dissolution of this compound in a large volume of water and subsequent treatment with aqueous potassium cyanide in the presence of ammonium chloride, an orange oil was obtained which was presumed to be the dihydropyridine (190):



The infra-red spectrum of the crude material showed two cyanide peaks at 2230 and 2220 cm^{-1} with two carbonyl absorptions at 1680 and 1625cm^{-1} due to the N-acetyl group and the bridging carbonyl group, respectively. The mass spectrum showed only peaks due to the fully aromatic nitrile (191) and for this reason it was felt that the presence of two discrete cyanide absorptions in the infra-red spectrum could be explained in terms of having a partially-aromatised product:

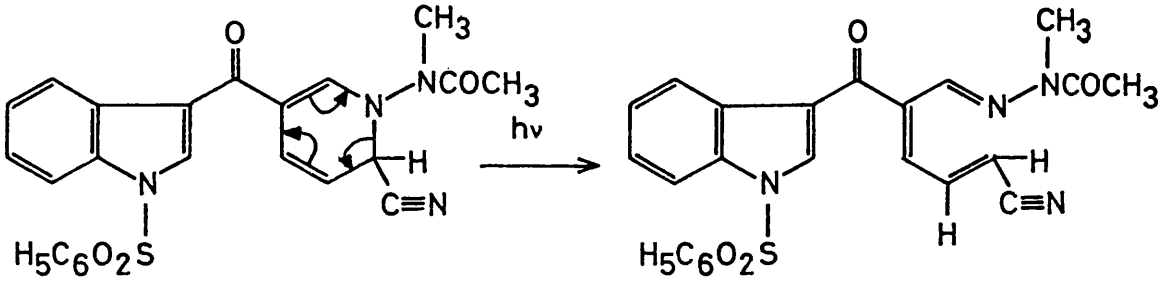


190 191

The ^1H nuclear magnetic resonance spectrum of this material was not particularly well-resolved and reflected the purity of the crude product. There was a three-proton singlet at δ1.94 due to the acetyl group and another three-proton singlet at δ3.31 due to the N-methyl group. A sharp one-proton singlet at δ7.28 was characteristic of the indole-2-proton but further interpretation of the complex aromatic signals was not possible.

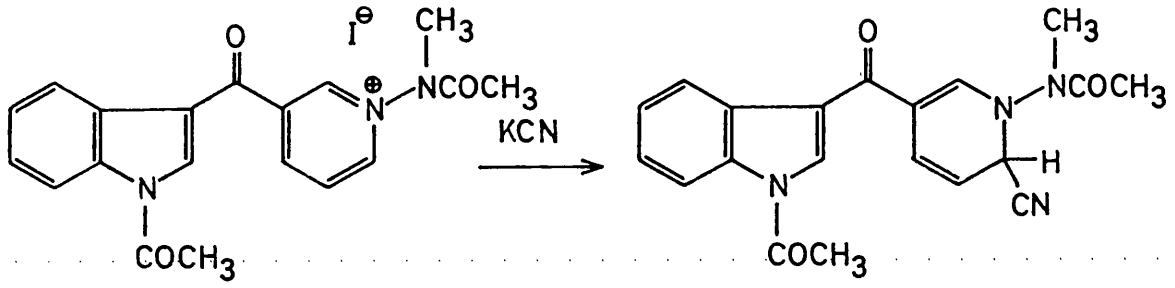
The oil was dissolved in ethanol and irradiated under a medium pressure ultra-violet source for about one hour and then the solvent was removed under vacuum to leave an orange oil which was chromatographed on a column of silica gel using chloroform as eluent. The first fractions were combined and the solvent removed under reduced pressure to give an oil which yielded an amorphous orange solid on trituration with ether. Comparison with the original orange oil on TLC showed that significant purification had

been achieved and that this material gave rise to a spot which was also present in the crude reaction product. The infra-red spectrum now showed only a single cyanide band at 2220cm^{-1}, but both carbonyl bands were still evident at the same wave numbers as before, indicating that the pyridine N-substituent had been retained and that aromatisation could not have taken place. In essence, the ^{1}H nuclear magnetic resonance spectrum was the same as for the starting material except that now an olefinic proton signal could be discerned at δ6.76. There were no low-field signals below δ8.6 which are so characteristic of the aromatic pyridine nucleus and these observations led to speculation that the cyanide group had been introduced at the 6-position rather than the 4-position, and that photolysis served only to ring-open the resultant dihydropyridine (192) rather than promoting its aromatisation.



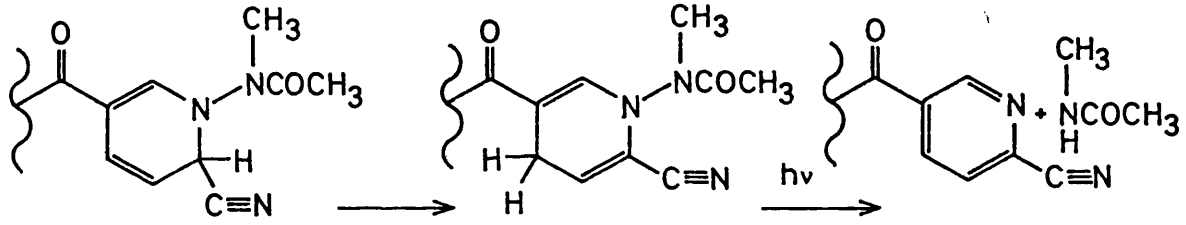
192 193

Such a process is not unprecedented, having been previously observed when the indole-N-acetyl analogue (194) was treated with aqueous potassium cyanide in chloroform solution[43]. In this case both the ring-opened product and the 4-cyanopyridine were obtained, and the relative proportions of each seemed to depend upon whether the pyridinium salt was dissolved in chloroform or water prior to the addition of the nucleophile:



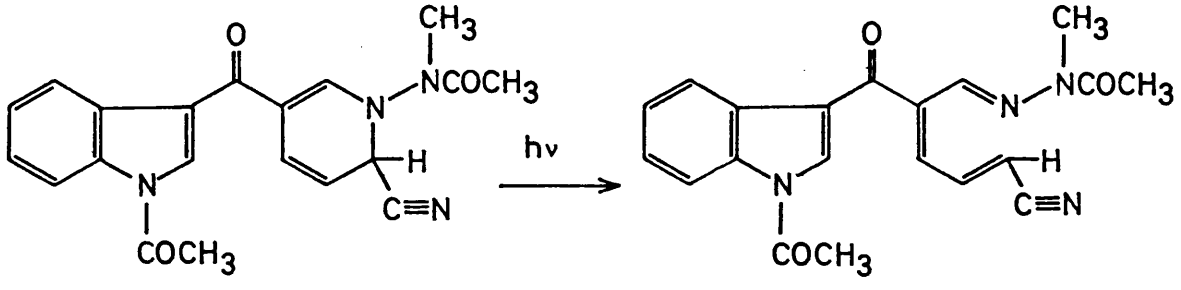
194 195

Originally it was supposed that, under the appropriate solvation conditions, the dihydropyridine (195) underwent prototropic change to the isomeric 1,4-dihydro compound (196) which is a much more favourable substrate for photolytic aromatisation to the 6-cyano-pyridine (197):



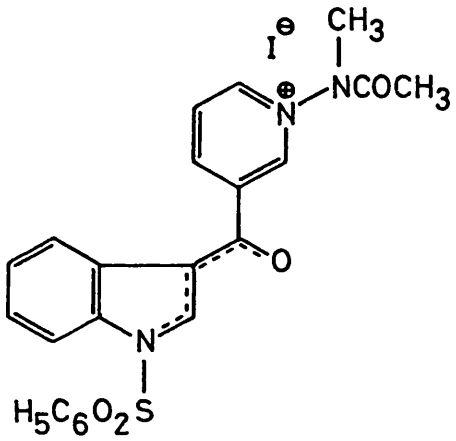
195 196 197

In less polar conditions the favourability of this trans-formation to the 1,4-dihydropyridine is not so great, and in this case photolysis gives the ring-opened compound (198), in direct analogy to the formation of (193):



195 198

When the indole nitrogen atom is substituted with a phenylsulphonyl group, as in the most recent work, the solvent appears to play a less important part in determining the position at which cyanide ion attacks the pyridine nucleus. Here the influence seems to be entirely steric and it is likely that the bridging carbonyl group gives the methiodide salt (189) a degree of rigidity which its methylene-bridged counterparts ((77), for example) lack:



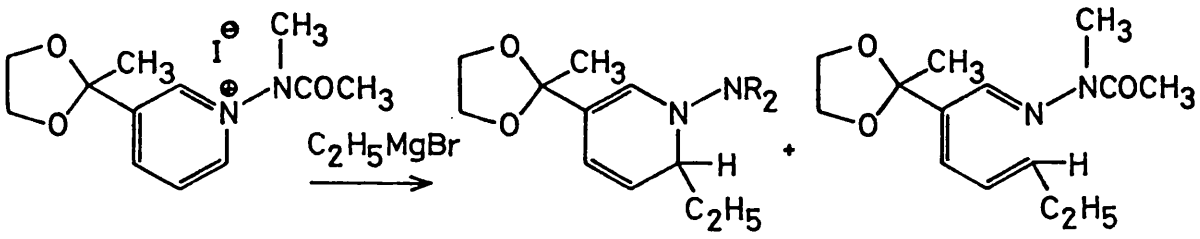
189

This is due to the vinylogous amide unit which imparts double bond character to the link between the indole and the carbonyl group. Just as simple amides have strong conformational preferences[99], a salt such as (189) might adopt the geometry indicated, in which the 6-position is now more accessible than in other cases where greater mobility is allowed.

In the case of simple amides, analysis of the ^1H nuclear magnetic resonance spectrum often allows a measurement of the relative amounts of E- and Z- isomers present. However, the spectra of the amides (189) and (194), together with those of the corresponding simple pyridines, show only one set of resonances per compound and thus it is not possible to analyse the problem in this way. This is unfortunate because it had been hoped that in changing the

indole N-substituent from an acetyl function to a phenylsulphonyl group, which resulted in a marked shift in the carbonyl stretching frequency in the infra-red spectrum (see page 62), the vinylogous amide character might have been reduced sufficiently to allow some interconversion and hence lead on to the desired cyanopyridine.

Possibly an answer to these speculations may only be achieved through crystallographic measurements, but in such circumstances it is important to note that the comparison between the situation in the solid state and that in solution may not be justified. Nevertheless, steric factors appear to be very influential in these reactions, for it has been observed that in the reaction between the acetal of 1-(N-acetyl-N-methylamino)-3-acetylpyridinium iodide (199) and ethylmagnesium bromide, both 6-ethyl-(200) and ring-opened products are obtained rather than the 4-substituted compound[100]:

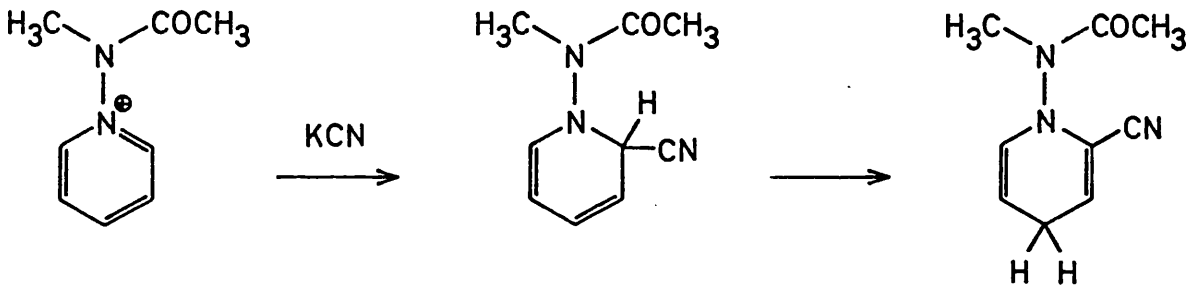


199 200

It seems that the rigid and bulky acetal function blocks access to both the 2- and 4- positions, allowing attack at the 6-position only.

A Japanese group [101,102] have claimed that 2-cyano- 1,2-dihydropyridine (202) was obtained from the reaction of cyanide ion with the salt (201), and that electron-withdrawing substituents in the 3-position of the pyridinium ring promoted addition at the 6-position. They also observed that reactions performed under

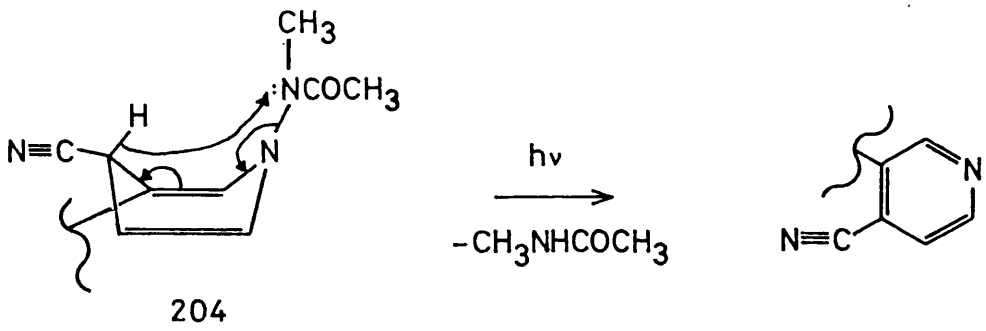
wholly aqueous conditions gave rise to attack at the 4-position whereas when less polar solvents were used considerable quantities of the 6-substituted product were obtained. It is to be expected that in a protonated solvent, rearrangement of the 1,2-dihydropyridine (202) takes place to give the 2-cyano-1,4-dihydro derivative (203) to facilitate easier aromatisation through loss of N-methylacetamide:



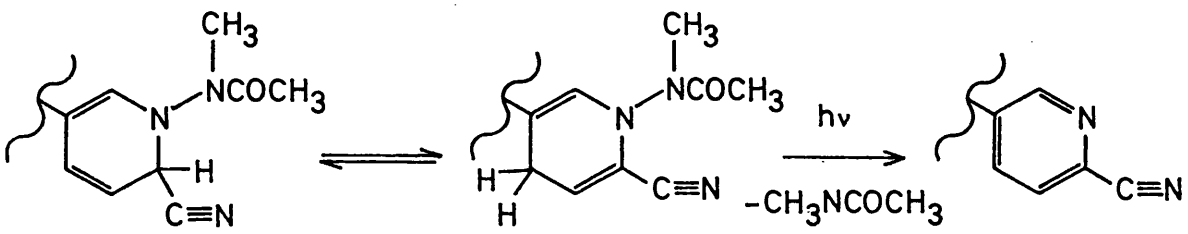
201 202 203

Sammes and Katritzky[103] have also commented upon the ring opening effect of cyanide ion in pyridinium salts of this type and they, too, note that the question of whether 4- or 6- attack occurs depends upon the solvent conditions, although their results are at variance with those of workers at Bath.

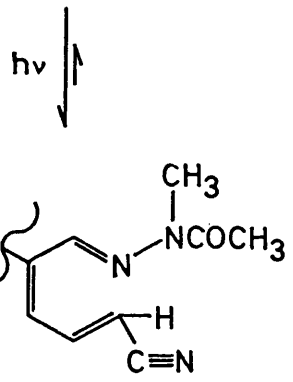
It is clear that addition at the 4- position readily leads onto the pyridine, for in a conformation such as (204) a concerted loss of N-methylacetamide is easily achieved. Before a similar loss can occur from the 6-adduct (205), a prototropic movement is required and it seems likely that such a change is in kinetic competition with a simple reverse electrocyclisation process, the latter being slightly favoured.



204



205

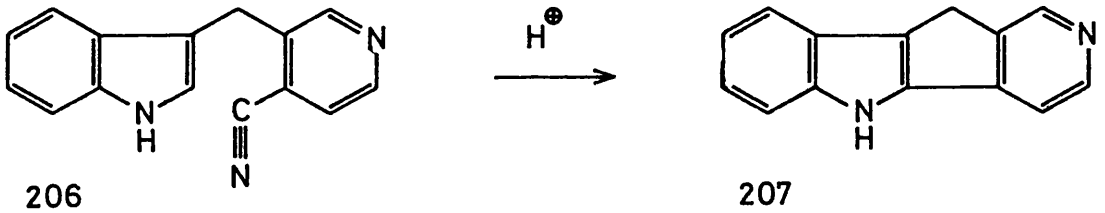


Driver[104] isolated some of the 6-cyanopyridine product from this reaction which suggests that the equilibrium is finely balanced.

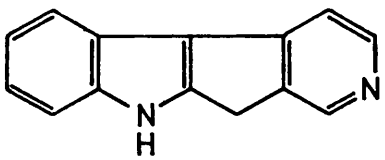
Further work in this area was postponed until such time as a suitable protecting group could be selected for the indole nitrogen atom which would not induce attack by cyanide ion at the 6-position of the pyridium ring. It would also have been valuable to minimise the influence of the ketone function by acetalisation in order to ascertain whether this type of group in the 3-position of the pyridium salt would promote attack in the 4- or 6- position by a nucleophile such as cyanide ion.

Attempts to prepare 10H-indolo [3,2-b]-2-azaindene (207):

During an earlier piece of work conducted at Bath[104] involving the methylene-bridged analogue (206) of the cyanopyridine (78a), an initial attempt to hydrolyse the nitrile function of this compound by treatment with boiling 2M hydrochloric acid gave rise to an unexpected product which was difficult to purify by recrystallisation. It was clear, however, that a new substance had been formed, since its spectral characteristics were quite different from those of the starting material. For instance, the infra-red spectrum displayed no evidence of a cyanide stretching absorption, and the mass spectrum showed a molecular ion peak at m/e 206 which was two units less than the picolylindole from which it was derived. The ^{1}H nuclear magnetic resonance spectrum comprised a two-proton singlet at δ3.8 due to the bridging methylene group, an indolic N-H signal at δ11.6, a singlet at δ8.8, a doublet at δ8.6 (J=5HZ) and two complex series of signals at δ7.7 and δ7.3ppm equivalent to three and two protons, respectively. Given this information, the compound was tentatively assigned the tetracyclic structure (207):

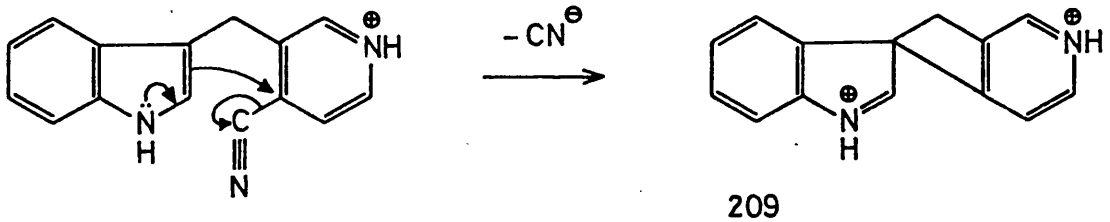


206 207



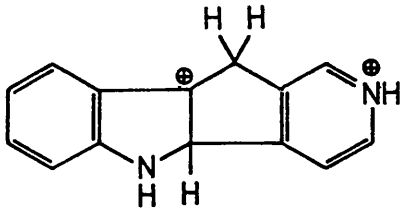
208

The isomeric structure (208) was considered less likely from a mechanistic point of view, for whilst it is true that substitution at the 2-position of an indole is commonly preceded by attack at C-3, with subsequent rearrangement, in this case the necessary intermediate (209) would have contained a highly-strained four-membered spiro unit:

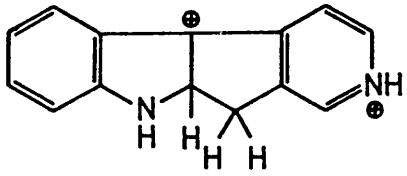


209

Furthermore, it was felt that rearrangement of this species in acid solution would favour the carbonium ion (210) rather than its counterpart (211), although under the conditions employed in this reaction, both would be destabilised:

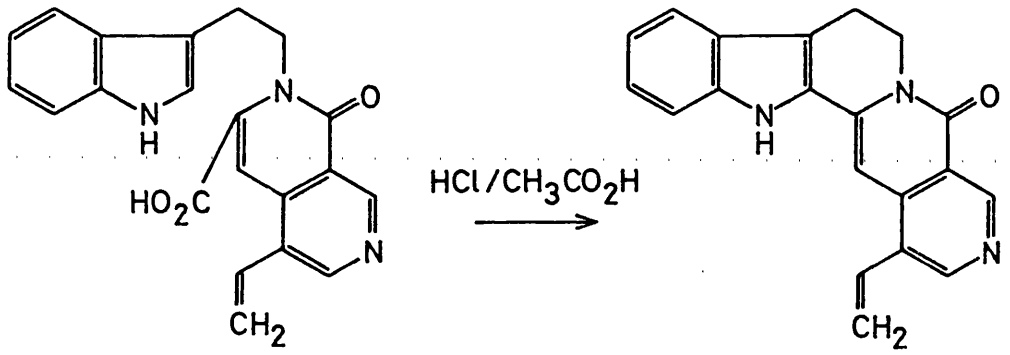


210



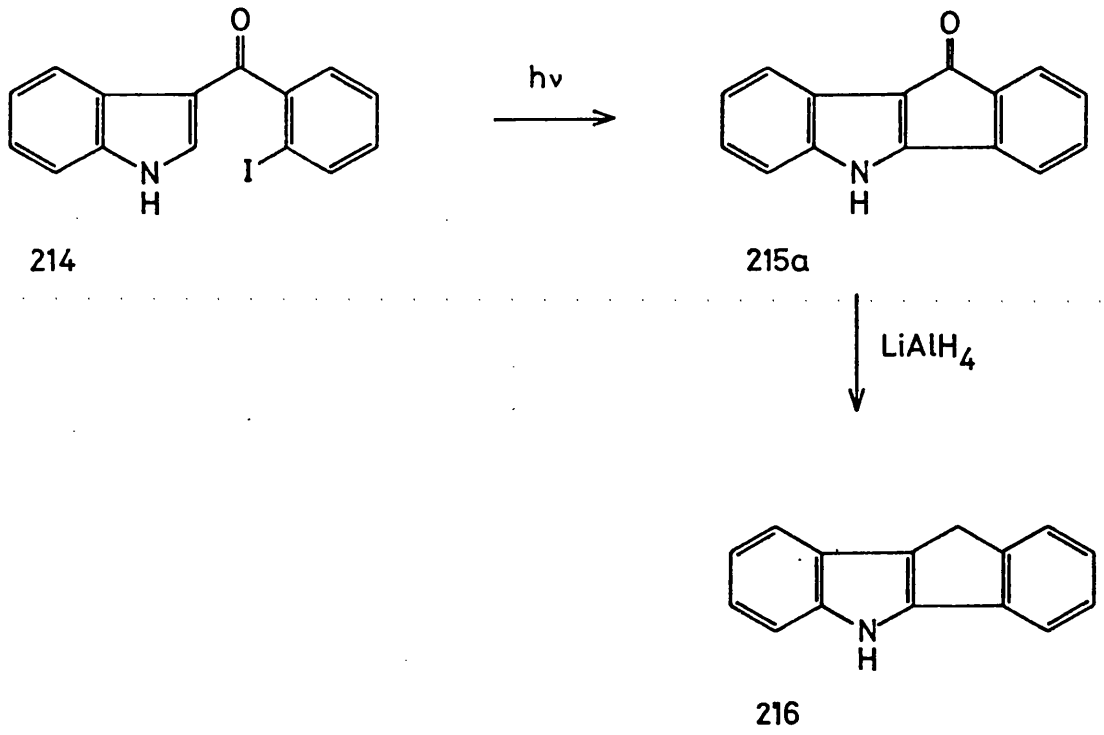
211

Direct attack at the indole-2-position gains further support from the work of Kametani et al.[105], who reported that the 7-aza-carbostyryl (212) formed the alkaloid (213) on treatment with a mixture of hydrochloric and acetic acids:



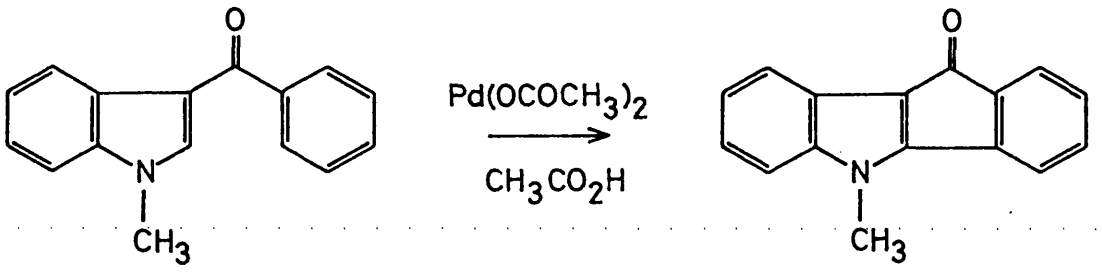
212 213

It has also been reported[106] that photolysis of 3-(2-iodobenzoyl) indole (214) gives indeno [1,2-b] indol-10(5H)-one (215a) and that reduction of this compound with lithium aluminium hydride results in the formation of 5,10-dihydroindeno [1,2-b] indole (216). A comparison between the ultra-violet spectra of this latter compound and the product obtained by Driver reveals that the two share many common features. However, it is likely that the isomer (208) will also have similar ultra-violet absorption characteristics, and a favourable comparison here was not considered sufficient evidence to be assertive about the structure of the unknown compound.



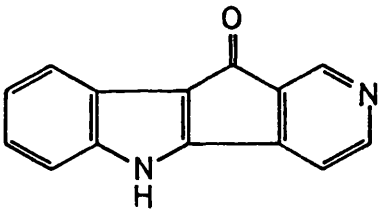
In order to obtain further structural evidence, attempts were made to acetylate the compound at the indole nitrogen atom, since it was anticipated that the deshielding effect of a carbonyl group upon the methylene bridge protons would be clearly noticeable in structure (208) but less marked in (207). Unfortunately such a derivative could not be obtained in the pure state and further work was postponed until an unequivocal synthesis of either or both of these isomers could be achieved.

More recently, Itahara and Sakakibara[107] reported the intramolecular ring closure of 3-benzoylindoles using palladium acetate. Treatment of 3-benzoyl-1-methylindole (217) in acetic acid with 0.5 molecular equivalents of palladium acetate gave 5-methyl-5,10-dihydroindeno[1,2-b] indol-10-one (218) in 60% yield.



217 218

This reaction was seen as an excellent model for the palladium acetate ring-closure of indol-3-yl-3-pyridyl methanone (111) to give the pyridine analogue (215b) of Carruthers' photolysis product (215a).



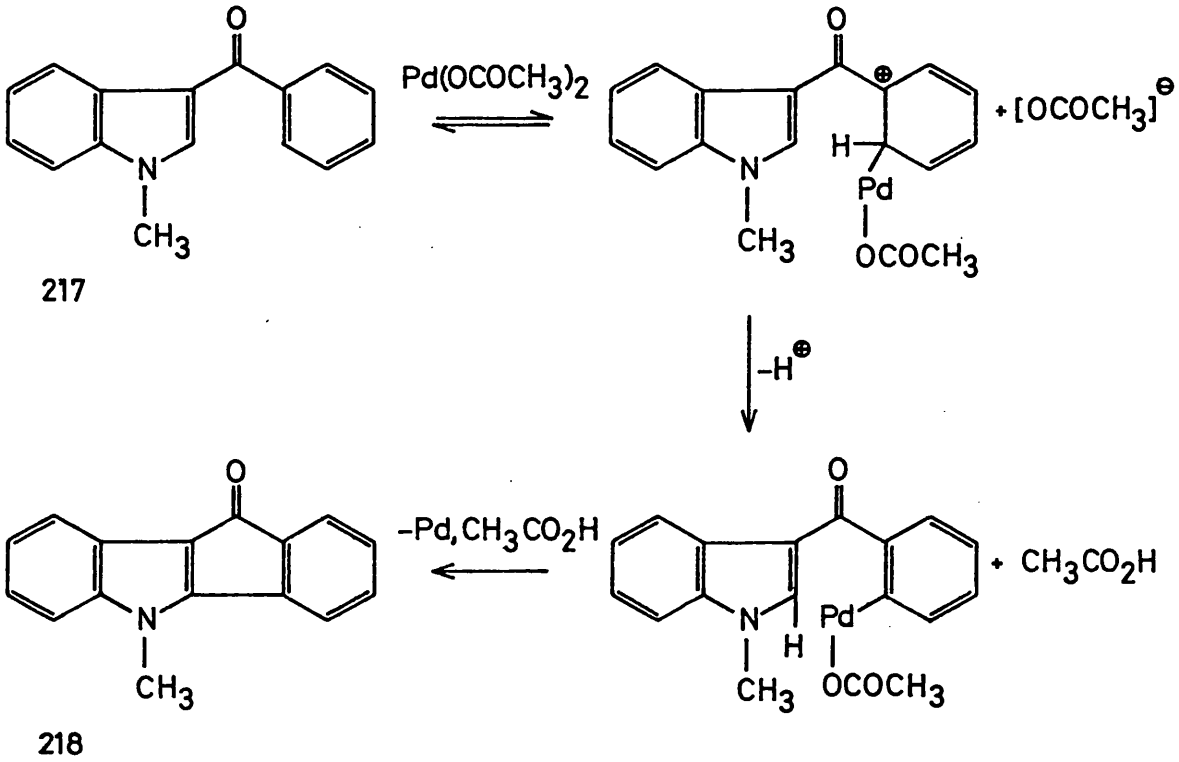
215b

Typically, indoles substituted with an acyl function at the 3-position give rise to ^{1}H nuclear magnetic resonance spectra in which one of the distinguishing characteristics is the position and multiplicity of the signal due to the indole C-4 proton. This appears as a double doublet (J_1=9HZ and J_2=5HZ) centred at δ8.2. The Japanese workers failed to comment upon this feature, and unfortunately the signal is masked in the pyridyl methanone (111). However, it was hoped to confirm the structure of the ring-closed product through decoupling experiments.

In an initial reaction, the ketone (111) was dissolved in

glacial acetic acid with a 0.5 molecular equivalent portion of palladium acetate and the mixture was heated for some forty hours under an inert atmosphere. Subsequent work-up and analysis of the product revealed that it was unchanged starting material, since both the infra-red and ^1H nuclear magnetic resonance spectra were superimposable upon those of the unreacted ketone, and it was feared that the basic nitrogen atom in the pyridine ring was too susceptible to palladation to allow the reaction to proceed as expected.

In the normal course of events, palladium acetate reacts with aromatic species in a manner closely resembling electrophilic substitution[108], with formation of an analogous σ-complex and subsequent loss of acetic acid to give the palladated species. Intramolecular ring closure is then achieved through elimination of a proton and the anion $[PdOCOCH_3]^{\ominus}$ so that during the reaction the metal becomes reduced to its zero oxidation state. This scheme is illustrated below for 3-benzoyl-1-methylindole (217):

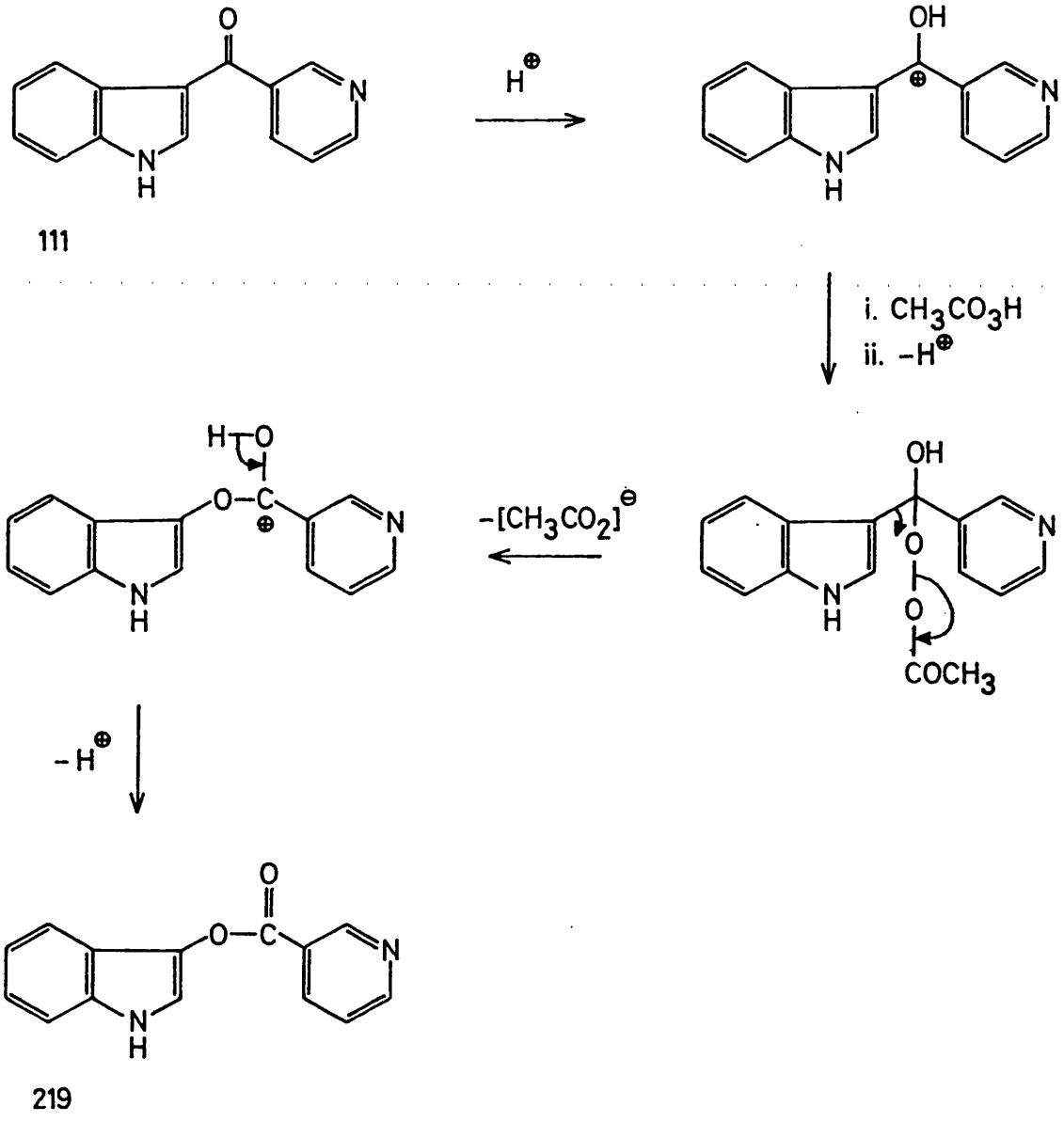


217

218

In order to overcome the problem of undesired nitrogen palladation it was decided to prepare the pyridine N-oxide of the methanone (111), but this proposal also resulted in unforeseen difficulties when put into practice. On treatment with hydrogen peroxide in glacial acetic acid at a constant 70^{o}, analysis by TLC indicated that a complex mixture had been obtained and that the reaction had not proceeded to completion. The product was flash-chromatographed on a column of silica gel, but with limited success since the only component which could be isolated in sufficient quantity to allow identification was unchanged starting ketone. A second fraction which showed two spots on TLC was submitted for mass spectral analysis, whereupon two molecular ion peaks were detected at m/e 238 and m/e 222. The latter peak was almost certainly due to the starting material again, but the first one was an interesting discovery since its value was 16 m/e units greater than the parent ketone, implying the inclusion of an extra oxygen atom. However, it was felt that pyridine N-oxide would not be detectable in the mass spectrometer except as the N-desubstituted compound, and this led to speculation of a Baeyer-Villiger type oxidation in which the ketone was converted to the ester (219) (see following page).

There was insufficient material to confirm this supposition by any other means and since it had become apparent that the conditions necessary for palladium acetate ring closure were quite stringent, it was decided to abandon this work in favour of a more pressing, though not totally unrelated problem.

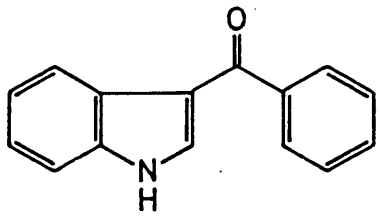


111

219

Subsequent work[109], aimed at establishing the importance of the indole-N-substituent and the electronic nature of the second ring system in determining whether ring closure occurs and at which position of the indole nucleus, has disclosed some interesting discoveries. In particular, it was found that the major product of the palladium acetate - catalysed cyclisation of 3-benzoylindole (220) was the 4-substituted derivative (221) and not the expected

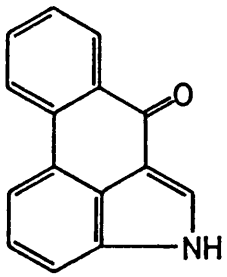
2-substituted compound (222). Itahara reported that cyclisation to the 4-position occurred only in systems which already possessed a substituent at the 2-position. It would appear that this is not true for indole systems which lack the indole-N-substituent:



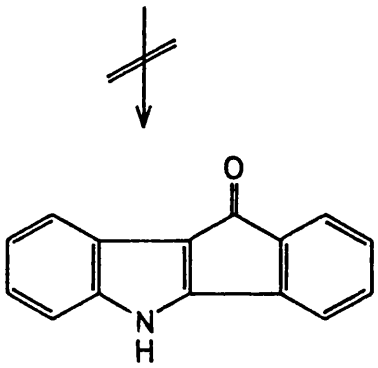
220

$Pd(OCOCH_3)_2$

CH_3CO_2H



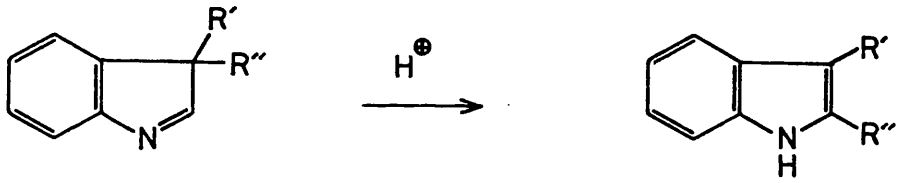
221



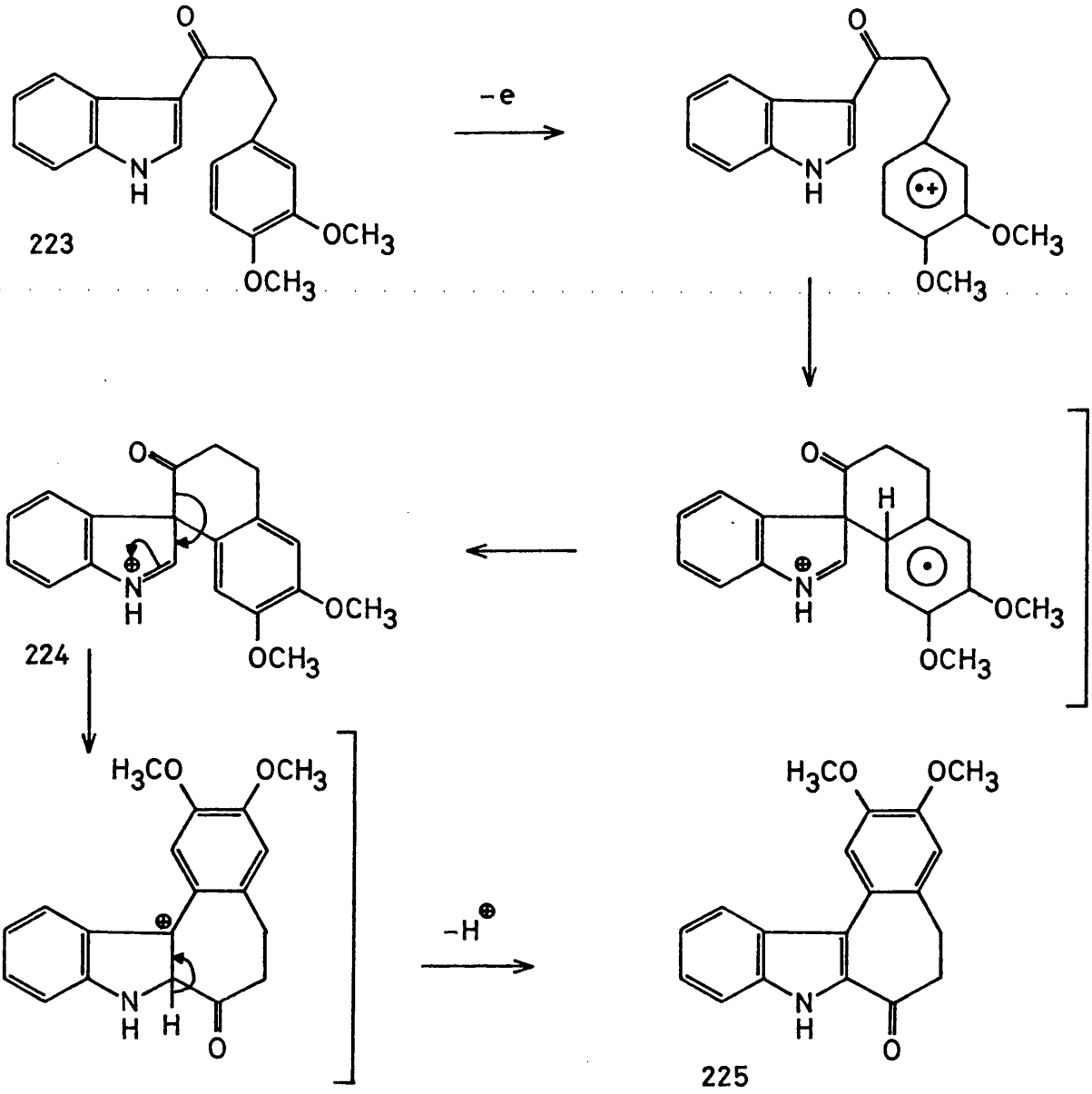
222

Ellipticine correlations:

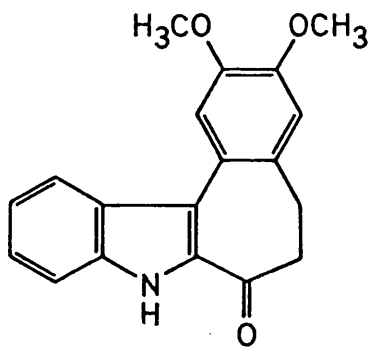
The concept of rearrangement of 3,3-dialkylindolenines is by no means unexplored. In 1968 Jackson and Smith[110] prepared a number of such compounds by the action of alkyl halides upon the Grignard derivatives of 3-alkylindoles and on subsequent acid treatment they observed that rearrangement occurred through migration of the higher alkyl group to give a single 2,3-dialkyl indole product:



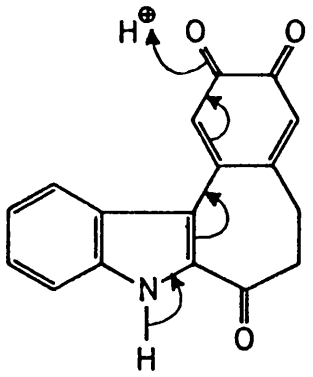
In the synthesis of more complex indoles this property frequently leads to problems in structural determination. For instance, the indole derivative (223) undergoes anodic oxidation at a potential of +1.35V (versus SCE) to give a tetracyclic product which has been formulated as the azaquinone (227)[111]. This is thought to arise by the mechanism illustrated on the following page:



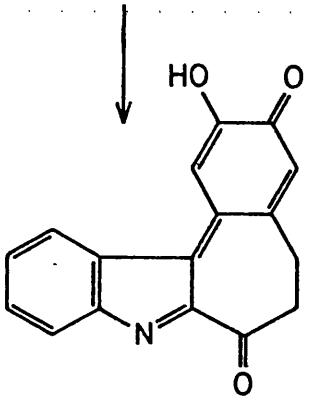
The anticipated product (225) is then further oxidised to the quinone (226) which is tautomeric with (227):



225

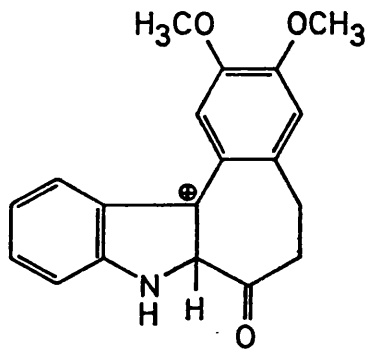


226

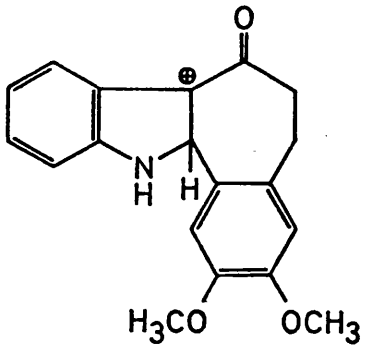


227

Spectral data are consistent with this structural assignment, but it should be emphasised that the proposed mechanism assumes attack of the indole unit at the 3-position by the initially-formed radical cation from the dimethyoxylated aryl ring system. Rearrangement of the spiro cation (224) takes place as shown in order that the newly-formed cation (228) may be stabilised by resonance. Rearrangement by the alternative mode would give rise to a cation (229) in which the positive charge is formally located next to a carbonyl group, and this would be strongly disfavoured:

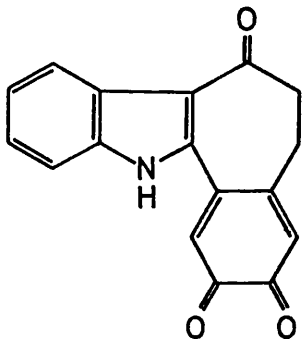


228



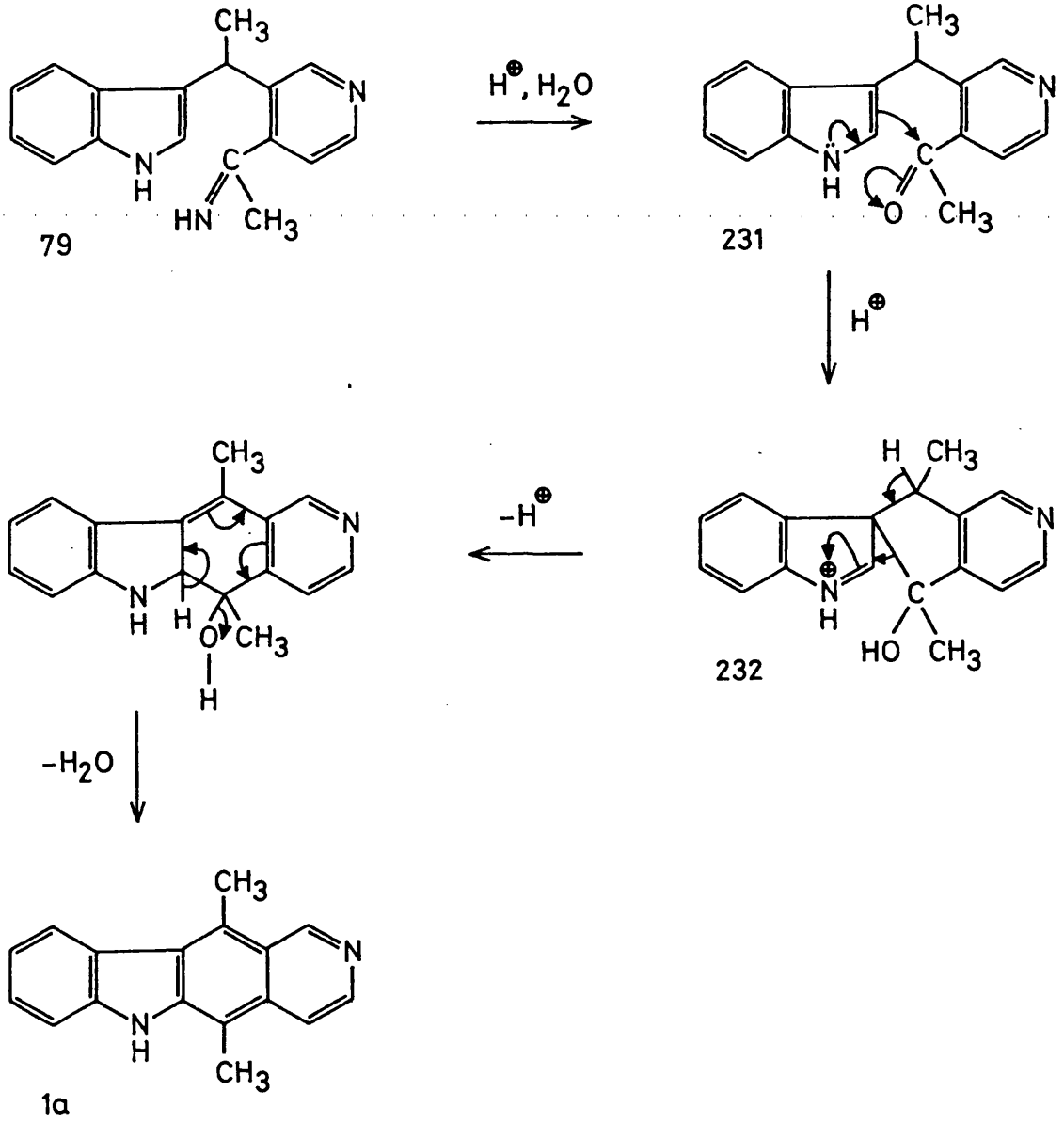
229

Nevertheless, the relative stabilities of these cations is not considered proof of structure (227). Furthermore, if direct cyclisation occurs at the 2-position of the indole nucleus, a product isomeric with (227) is to be expected, in this case the orthoquinone (230).

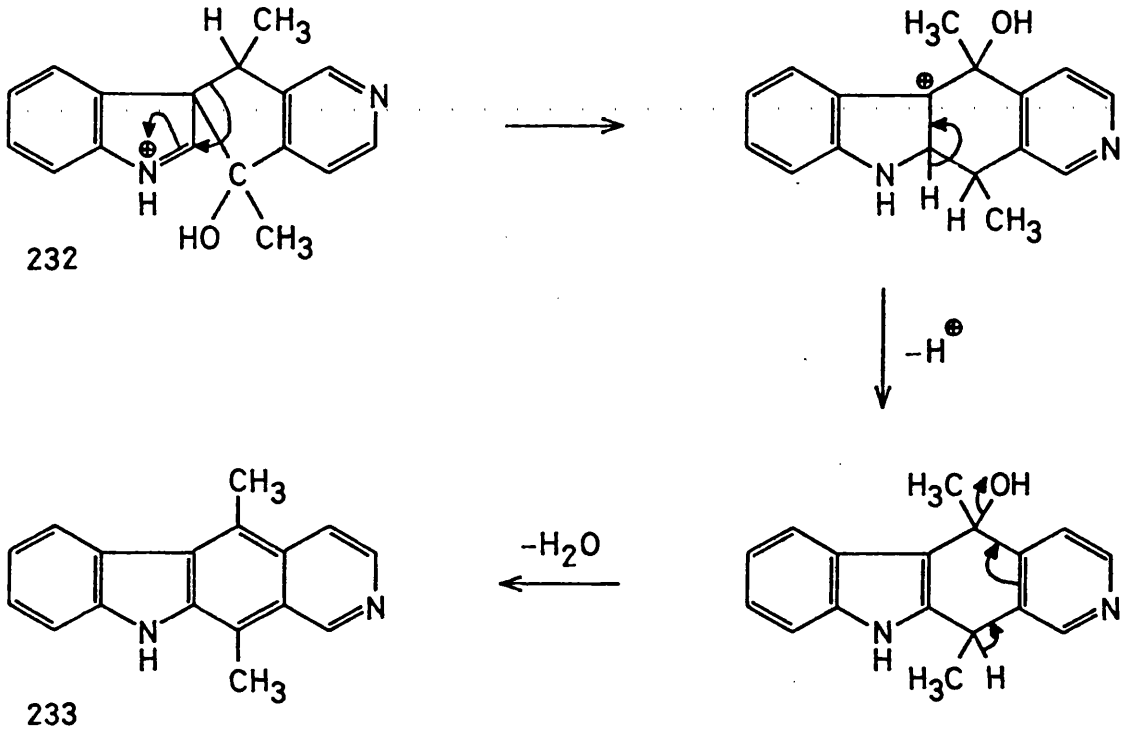


230

This phenomenon has apparently never manifested itself in syntheses of ellipticine derivatives where formation of the tetracyclic skeleton is achieved through cyclisation of the C ring to an indole nucleus. For example, in the acid-catalysed cyclisation of the imine (79), which presumably takes place via the acetyl species (231), ellipticine (1a) is isolated as the sole product in near-quantitative yield. There is no evidence for the presence of the isomeric tetracycle (233), and should the spiro intermediate (232) be involved, the implication is that one of the bonds is preferentially cleaved, resulting in regiospecific rearrangement.


CH3
N
H
C
HN
CH3
79
H⊕, H2O
CH3
N
H
C
O
CH3
231
H⊕
H
CH3
N
H
C
HO
CH3
232
-H⊕
CH3
N
H
H
O
CH3
H
-H2O
CH3
N
H
CH3
1a

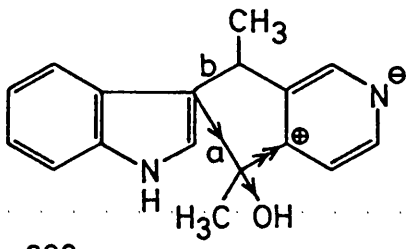
The alternative rearrangement may be envisaged as follows:



232

233

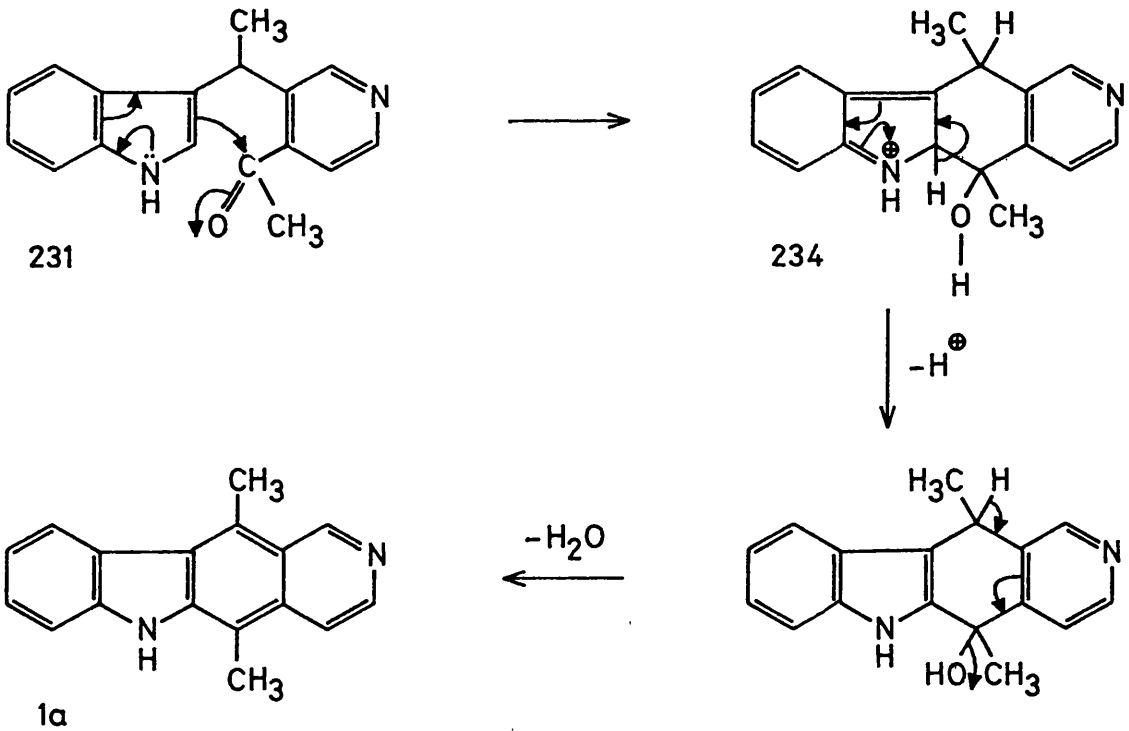
The fact that formation of the pyrido [4,3-b] carbazole is favoured exclusively over formation of the pyrido [3,4-b] carbazole clearly indicates that one of the bonds in the spiro intermediate is weaker than the other. This may be explained in terms of a simple resonance description of the pyridine unit: The 4-position of pyridine is a point of low electron density and therefore substituents here are subject to a positive inductive effect and this may significantly diminish the strength of bond "a" in the spiro intermediate (232). Such an effect is also reinforced by the proximity of the hydroxyl oxygen atom:



232

This is not true for substituents in the 3-position of pyridine, and hence bond "b" remains largely unaffected.

Conversely, the regiospecific formation of ellipticine may be seen as evidence for direct attack at the 2-position of the indole unit, thus:



231 234 1a

One argument against this proposal is that formation of the intermediate (234) results in a disruption of the aromatic character of the A ring and thus the activation energy for this facile cyclisation process is raised. However, since the reaction is so readily carried out, using dilute acetic acid at 70^{o}, it is obvious that the apparent activation barrier is very low. Thus direct attack at the 2-position of the indole unit seems less probable.

Whatever the true order of events, it is apparent that the alternatives are strongly influenced by the electronic configuration of the indole unit. As more groups of workers are attempting the synthesis of ellipticine derivatives bearing a variety of substituents in ring A by the type of ring closure reaction illustrated above, it is important that spectrometric data for the pyrido [4,3-b] carbazole system should be well-defined.

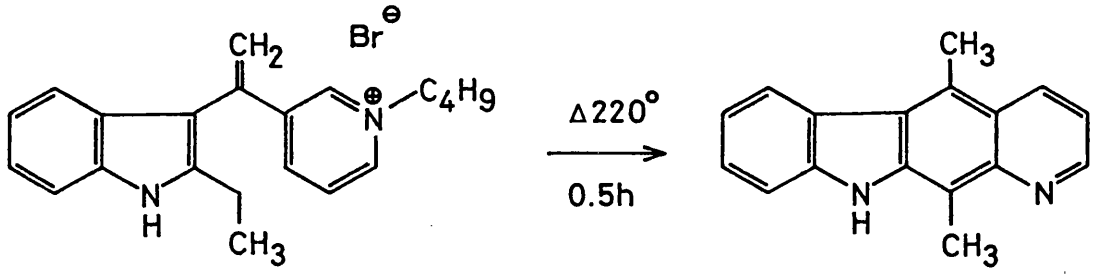
To dispel any doubts about the regiospecificity of the ring closure reaction it was decided to compare a sample of ellipticine prepared in this laboratory, using the method described previously, with a sample kindly donated by the Swedish team of Bergman and Carlsson. The Swedish compound had been synthesised according to the procedure outlined on page 40 and had been purified by sublimation.

Initial tests by TLC showed no discernible differences in retention indices between the samples in a variety of solvent systems and on different support materials. Similar results were achieved for the more stringent spectroscopic analyses, wherein the infra-red, ultra-violet and mass spectra were initially indistinguishable. The ^{1}H nuclear magnetic resonance spectra recorded in

dimethylsulphoxide solution indicated that the Swedish material had a slightly greater water content, but in all other respects the traces were coincidental.

However, the ^{13}C nuclear magnetic resonance spectra, also recorded in dimethylsulphoxide to facilitate easy comparison with reported data[112,113], were markedly different, an observation which was difficult to rationalise. Whilst it is true that the two previously published sets of chemical shift values are not entirely in agreement with each other (for comparison, see Table 2), the figures at least follow the same trend with respect to their relative positions, and have the same multiplicities in the off-frequency partially-decoupled spectrum. Assignment of the chemical shift values to the carbon skeleton of ellipticine is a matter open to some debate, but the Swedish sample gave a series of chemical shift values which bore little relation to the expected figures.

At first it seemed as if the anomalous spectrum was due to the isomer (235), since in the synthesis of ellipticine by pyrolysis of the N-alkylated pyridine precursor (85), the Swedes report that the 6H-pyrido [2,3-b] carbazole (235) is obtained on slow heating, whilst ellipticine is the predominant product when heating is rapid[46].



85 235

However, this possibility was precluded when a re-examination of the other spectral parameters mentioned previously clearly showed that the two ellipticine samples were identical in every respect and that no organic impurity was present. This led to speculation about the presence of an unknown paramagnetic impurity, and in an attempt to confirm this supposition, an atomic emission spectrum of the Swedish material was obtained. Inspection revealed that metal ions such as iron and manganese were indeed present in the sample, but since the concentration levels were only slightly greater than normal background readings, the level of contamination was not considered sufficiently high to account for the observed differences in the ^{13}C chemical shift values.

Table 2. Ellipticine ^{13}C chemical shift assignments

Carbon	American[112]	French[113]	British	Swedish
1	149.5	152.9	149.7	143.5
3	140.4	140.9	140.5	143.1
4	115.7	115.8	123.8	142.0
4a	140.9	132.3	132.5	133.1
5	123.0	107.9	110.7	132.1
5a	142.6	140.4	140.4	128.0
6a	132.4	142.6	142.7	127.8
7	110.6	110.5	107.9	124.9
8	127.0	127.0	115.8	123.7
9	119.1	119.1	127.1	121.7
10	123.7	123.6	119.1	120.1
10a	127.9	123.3	123.3	119.2
10b	107.9	121.9	121.9	118.7
11	123.3	123.0	123.1	111.1
11a	121.9	123.3	126.9	109.4
12	14.2	14.2	14.3	14.3
13	11.8	11.8	11.9	11.5

Chemical shift values are measured in parts per million downfield from TMS. The figures in the fifth column are in order of decreasing chemical shift.

Analysis by electron spin resonance, on the other hand, resulted in a singlet absorption at 2.0063 gauss, an observation which is consistent with the presence of some kind of oxy-radical[114]. The peak was only evident at the limit of instrument detection and showed no hyperfine splitting. It was speculated that the radical species may have arisen from the ellipticinium N-oxide, since to effect cyclisation, the Swedish workers had heated their precursor in a luminous Bunsen flame at a temperature in excess of 300^{o} in the presence of air. However, this does not adequately explain why the effect of the radical species is not manifest in the ^{1}H nuclear magnetic resonance spectrum and as yet this problem remains unresolved.

It was apparent that the contaminant was present only in a very low concentration, and simply by recrystallising the Swedish sample from methanol all traces could be removed so that the chemical shifts of the signals in the ^{13}C nuclear magnetic resonance spectrum of the purified compound were in almost complete agreement with those in the spectrum of the material prepared in this laboratory.

Having finally established the authenticity of both ellipticine samples,an attempt was now made at assigning the chemical shift values. This exercise was undertaken by comparison of the ellipticine data with model compounds from the literature such as indole, carbazole, isoquinoline and pyridine. The initial problem here was the interpretation of the ^{13}C nuclear magnetic resonance spectrum of 1,4-dimethylcarbazole, for although the French team of Ahond, Poupat and Potier claim to have fully characterised this compound, it was felt that their ^{13}C nuclear magnetic resonance assignments were not compatible with other published data[115].

In aliphatic systems the substitution of a hydrogen atom by a methyl group typically causes a shift downfield from TMS of some 9ppm, an observation which is termed the " α-effect". A similar trend is apparent for the substitution of a β-hydrogen atom by a methyl group and it is perhaps surprising that the magnitude of the downfield shift in the β-effect should be comparable to the α-effect. Methyl substitution of hydrogen atoms in the γ-position also produces a consistent change in the carbon absorption. This time, however, it is a shielding effect and the chemical shift is moved upfield by about 2.5ppm. The γ-effect appears to be primarily operative through space rather than along the bonds of the molecule.

In aromatic compounds a similar trend is observed, although the change in chemical shift value is smaller in magnitude. It has been suggested that changes in the local π-electron densities are instrumental in determining the ^{13}C shieldings in aromatic systems[116] and thus it was felt that the unsubstituted benzenoid ring in 1,4-dimethylcarbazole would remain largely unperturbed compared with carbazole itself. It was then anticipated that methyl substituents would tend to deshield all of the remaining carbon centres, which led to the interpretation illustrated below and laid out in Table 3.

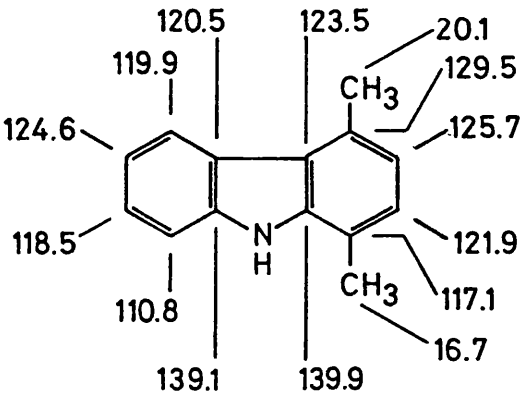


Table 3 ^{13}C Chemical shift assignments for carbazole and 1,4-dimethylcarbazole

Carbon	Carbazole[117]	1,4-Dimethylcarbazole	1,4-Dimethylcarbazole*
1	110.9 d	117.1 s	117.1 s
2	118.4 d	121.9 d	126.2 d
3	125.4 d	125.7 d	121.0 d
4	120.0 d	129.5 s	130.9 s
4a	122.4 s	123.5 s	121.5 s
4b	122.4 s	120.5 s	124.6 s
5	120.0 d	119.9 d	122.6 d
6	125.4 d	124.6 d	119.5 d
7	118.4 d	118.5 d	125.1 d
8	110.9 d	110.8 d	110.6 d
8a	139.7 s	139.1 s	138.8 s
9a	139.7 s	139.9 s	139.5 s
10		16.7 q	q
11		20.1 q	q

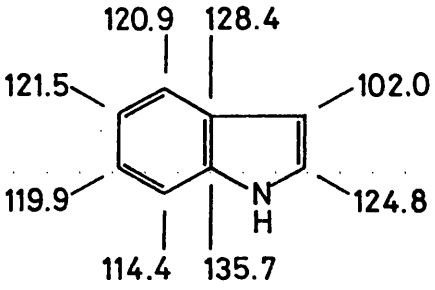
*Interpretation according to Ahond, Poupat and Potier[113].
The letters after the chemical shift values denote the multiplicity of the signal in the off-frequency partially-decoupled spectrum, wherein s = singlet; d = doublet and q = quartet

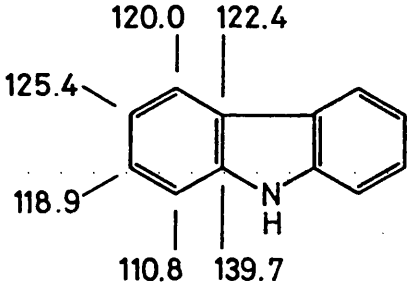
Having performed this operation, the interpretation of the ellipticine spectrum became much more straightforward since it involved the consideration of values from 1,4-dimethylcarbazole and pyridine. Certain resonances were easy to assign as they had occurred in many previous samples interpreted at Bath and were by now familiar signals. The seven tertiary carbon centres were clearly discernible as doublets in the off-frequency partially-decoupled spectrum and six of these were readily assigned to the ellipticine skeleton. For example, in pyridine the two carbon centres adjacent to the heteroatom resonate at 149.9 ppm. so the two lowest field doublets were designated as C-1 and C-3 in ellipticine. Similarly, in indole the assignments for C-4 to C-7 inclusive follow the trend C-7<C-6<C-4<C-5 in order

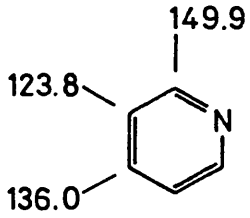
of increasing chemical shift measured downfield from TMS.

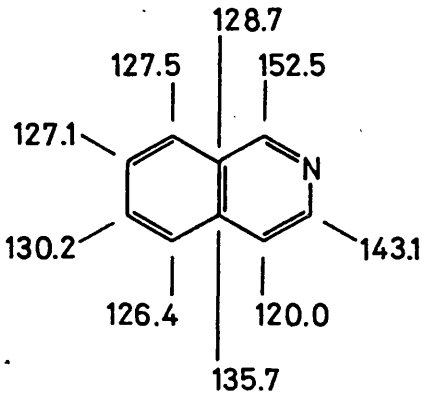
In ellipticine this sequence is reflected in the values 110.7<115.8<119.1<127.1 ppm for C-7, C-8, C-10 and C-9 respectively. The remaining doublet, which corresponds to the signal at 123.8 ppm in the proton-decoupled spectrum, was assigned to C-4 which is in excellent agreement with the value of 123.7 ppm reported for the C-3 resonance in pyridine itself.

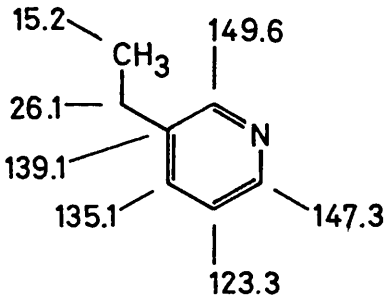
Some of the quarternary carbon resonances were more difficult to assign where there was no direct relation with a known compound. In particular this applied to the 11-position in ellipticine which could best be compared with the 8-position in 5,8-dimethylisoquinoline, a compound for which ^{13}C nuclear magnetic resonance data have not yet been reported. Nevertheless analogies could be drawn for most of the signals in a manner which is best understood by reference to Table 4 and the model systems illustrated on page 123.


120.9
128.4
121.5
102.0
119.9
N
H
124.8
114.4
135.7


120.0
122.4
125.4
118.9
N
H
110.8
139.7


149.9
123.8
N
136.0


128.7
127.5
152.5
127.1
N
130.2
143.1
126.4
120.0
135.7


15.2
CH3
149.6
26.1
N
139.1
135.1
147.3
123.3

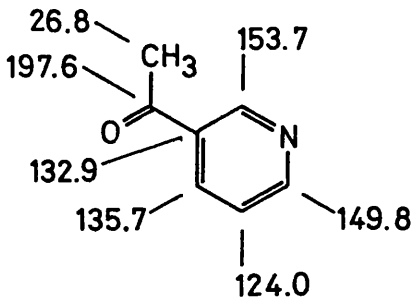

26.8
CH3
153.7
197.6
O
N
132.9
135.7
149.8
124.0

Table 4. Correlation data for ellipticine ^{13}C chemical shift values compared with model systems

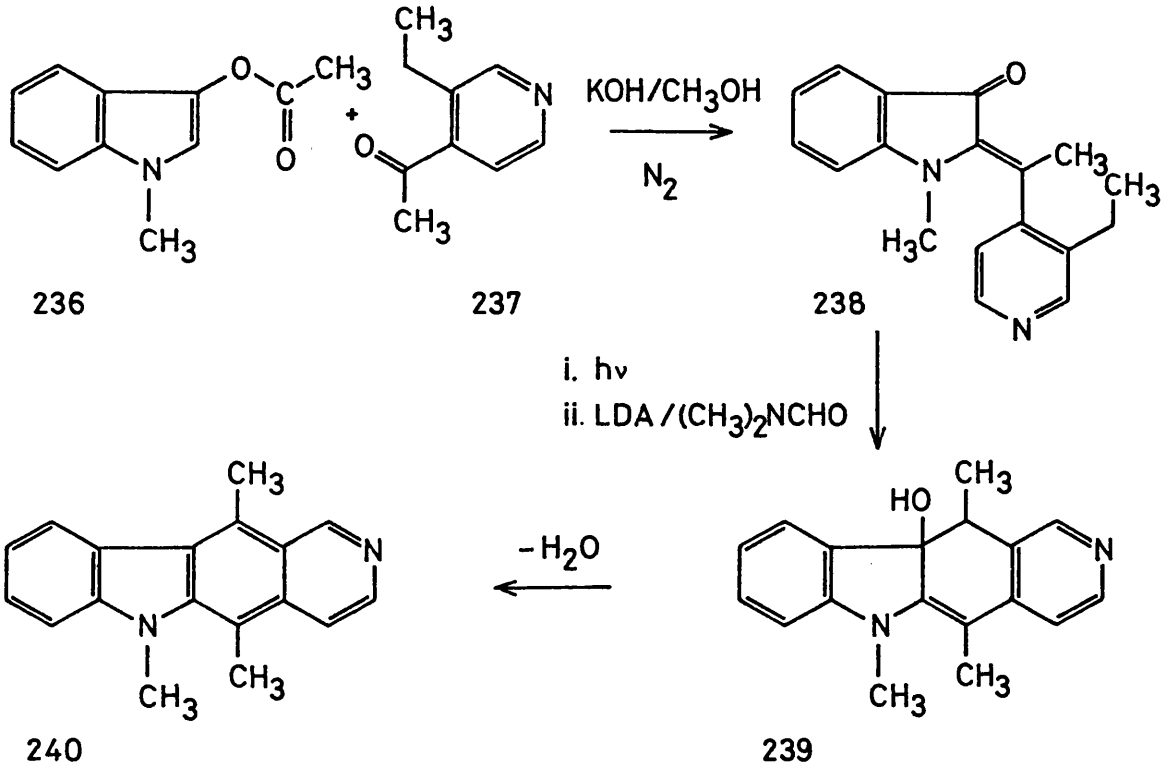
Carbon	Model, position and chemical shift		Inference and possible values	
4a	isoquinoline, 4a	135.7	low field signal	132.5 140.4 142.7
5	dimethylcarbazole,1	117.1	high field signal	107.9 121.9
5a	dimethylcarbazole,9a	139.9	low field signal	
6a	dimethylcarbazole, 8a	139.1	low field signal	
10a	dimethylcarbazole,4b	120.5	intermediate field signal	123.1 123.3
10b	dimethylcarbazole,4a	123.5	intermediate field signal	
11	dimethylcarbazole, 4	129.5	-	
11a	isoquinoline, 8a	128.7	-	

Chemical shift values are quoted in ppm measured downfield from TMS

Clearly ^{13}C chemical shift values for ellipticine are open to a variety of interpretations until such time as the effect of substituent groups in the pyrido [4,3-b] carbazole system can be reliably predicted. The chemical shift of carbon centres bearing a hydrogen atom could be unequivocally determined from the ^{13}C nuclear magnetic resonance spectra of the corresponding deuterium analogues: In such cases there would be no carbon-hydrogen coupling in the off-frequency partially-decoupled spectrum. However, the preparation of a series of such compounds would doubtless be a lengthy synthetic exercise and is beyond the scope of this work.

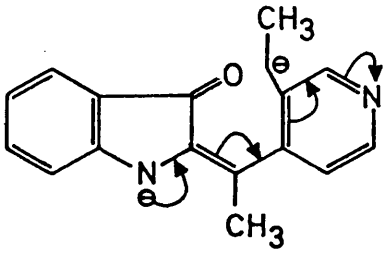
Synthetic efforts towards 6-methylellipticine (240):

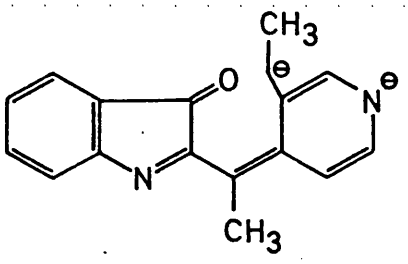
The proposed synthesis of 6-methylellipticine (240) using 1-methylindol-3-ol acetate (236) and 4-acetyl-3-ethylpyridine (237) as starting materials seemed attractive because it was anticipated that ring closure of the indoxylidene (238) could be achieved under mild conditions. In addition it was felt that dehydration of the precursor (239) might occur spontaneously to give the aromatic tetracycle in a single step, thereby dispensing with the need to isolate and purify the intermediate.



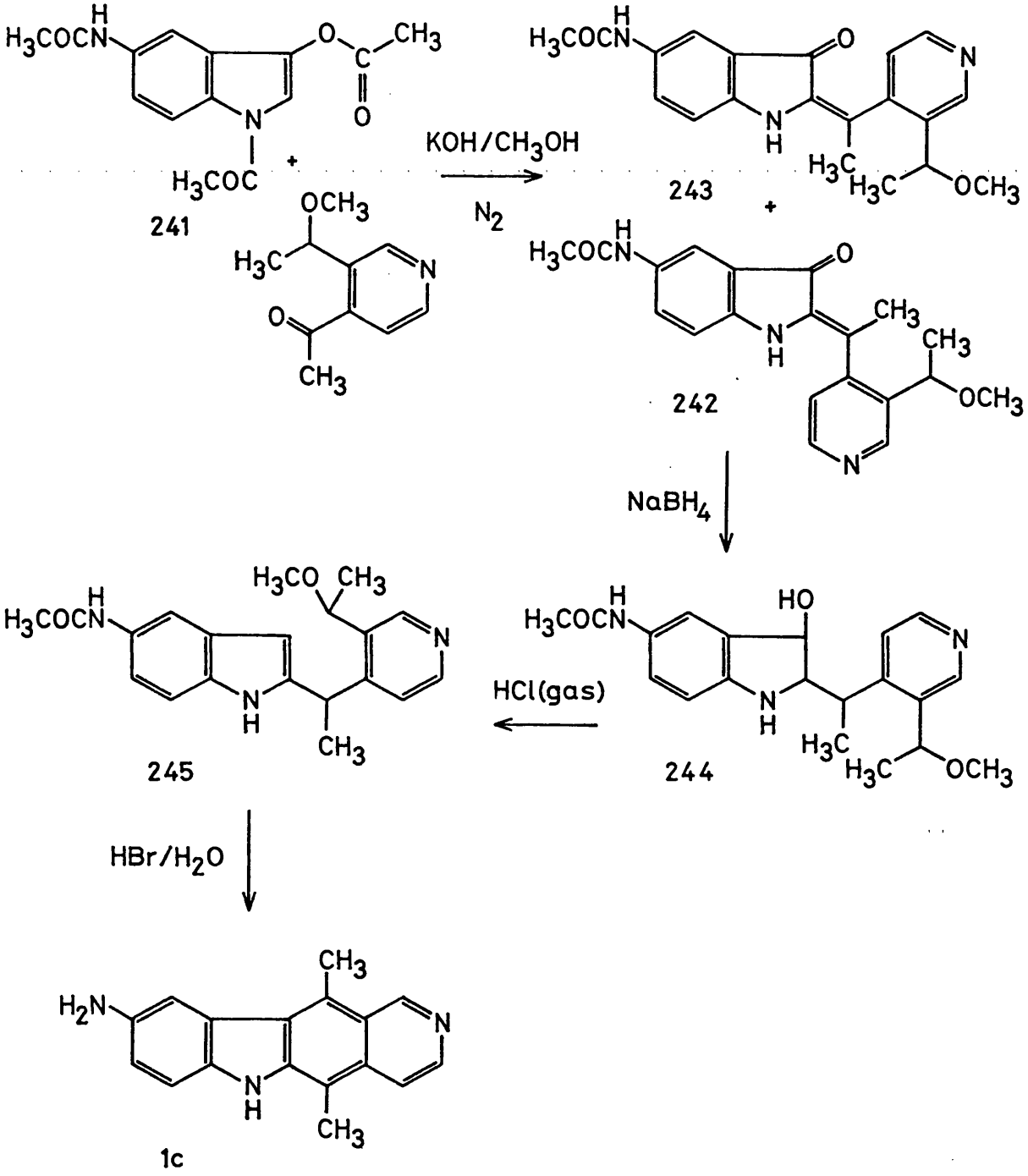
236 237 238 239 240

Cyclisation of the indoxylidene (238) would be promoted by strong base to generate a carbanion in the ethyl side chain in much the same way as picolyl anions may be obtained from the picolines. Thus it was necessary to protect the indole nitrogen atom with a methyl substituent in order to prevent removal of a proton here, with subsequent deactivation of the pyridine nucleus:

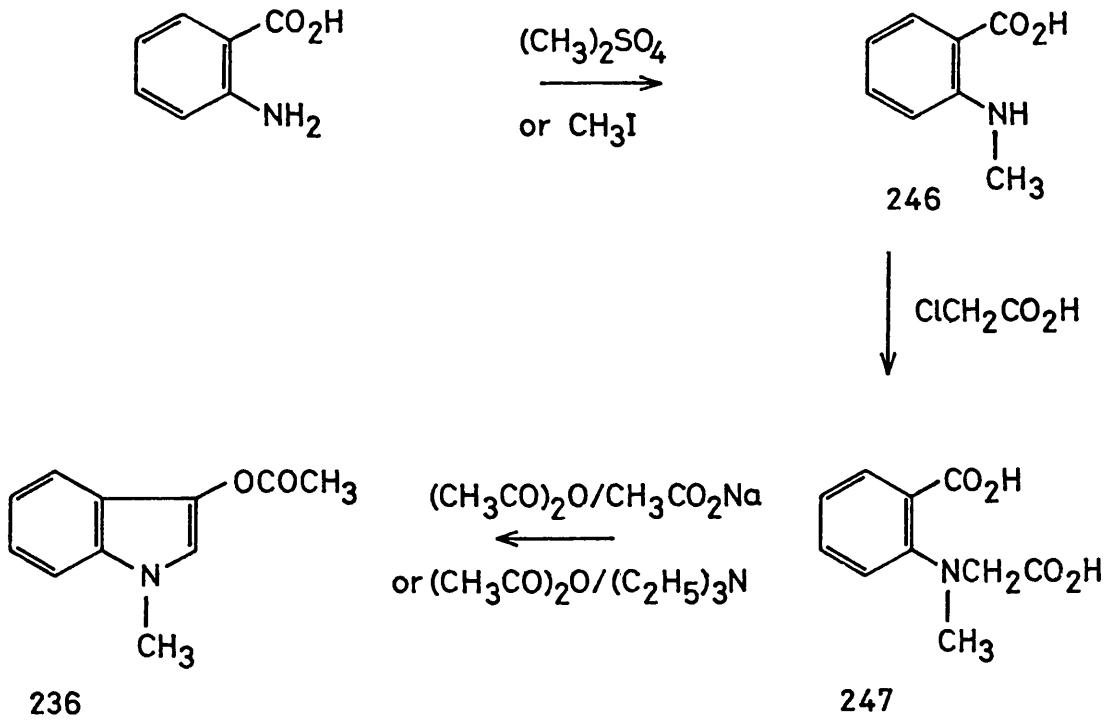




In previous work[32] along similar lines, 5-acetamido-1-acetyl-indol-3-ol acetate (241) was successfully condensed with 4-acetyl-3-(1-methoxy) ethylpyridine (31) to give the alkylidene indolinone as a mixture of E- and Z- isomers, (242) and (243). Without separation, the mixture was treated with sodium borohydride in boiling ethanol to give the hydroxyindoline (244), which, on treatment with hydrogen chloride, afforded the indole (245). This product was heated under reflux for eighteen hours in 60% aqueous hydrobromic acid to effect cyclisation to 9-aminoellipticine (1c,R=NH_2). Unfortunately, the forcing conditions employed here to bring about ring closure also encourage loss of the substituent in the benzenoid ring (see Scheme 10, page 25) so that the required product is contaminated with ellipticine and the yield is significantly diminished.


241
KOH/CH3OH
N2
243
242
NaBH4
HCl(gas)
244
245
HBr/H2O
1c

1-Methylindol-3-ol acetate (236) was prepared from anthranilic acid which was first methylated using either dimethyl sulphate or freshly distilled iodomethane in aqueous conditions, there being no appreciable difference in the yield of N-methylanthranilic acid (246) whichever compound was employed as the alkylating agent. Isolation of the product was dependent on its solubility in aqueous solution relative to the starting material and N,N-dimethylanthranilic acid. The compound was then reacted with chloroacetic acid to give the glycine derivative (247) which was cyclised to the desired product by refluxing with acetic anhydride in the presence of base:

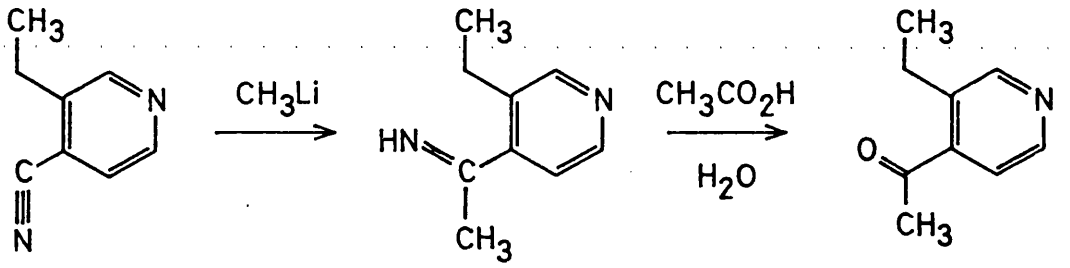


Two methods were compared in the preparation of N-methylphenylglycine-o-carboxylic acid (247): Initially, N-methylanthranilic acid was heated at reflux together with chloroacetic acid and sodium hydrogen carbonate in aqueous solution for twenty-four hours, and although it is claimed[118] that the yield of this reaction is enhanced in the presence of a catalytic amount of copper powder, the isolated yield was a disappointing 46%. The preferred method was an incubation technique in which separate aqueous solutions of N-methylanthranilic acid and chloroacetic acid, neutralised with sodium hydroxide and sodium carbonate respectively, were equilibrated at 40^{o} in a thermostatic water bath, then rapidly mixed and left to stand at the elevated temperature overnight. The resulting mixture gave an almost solid mass which was dissolved in a large volume of water and acidified to give the glycine derivative as a light tan precipitate in a much-improved yield of 75%.

Ring closure to the indoxyl ester was also effected by a variety of methods. Many other workers[119-121] favour the use of sodium acetate as the base in this reaction, and claim product yields of between 62 and 66%. It was found, however, that by using triethylamine the yield could be consistently increased to just over 70% and consequently this became the reagent of choice.

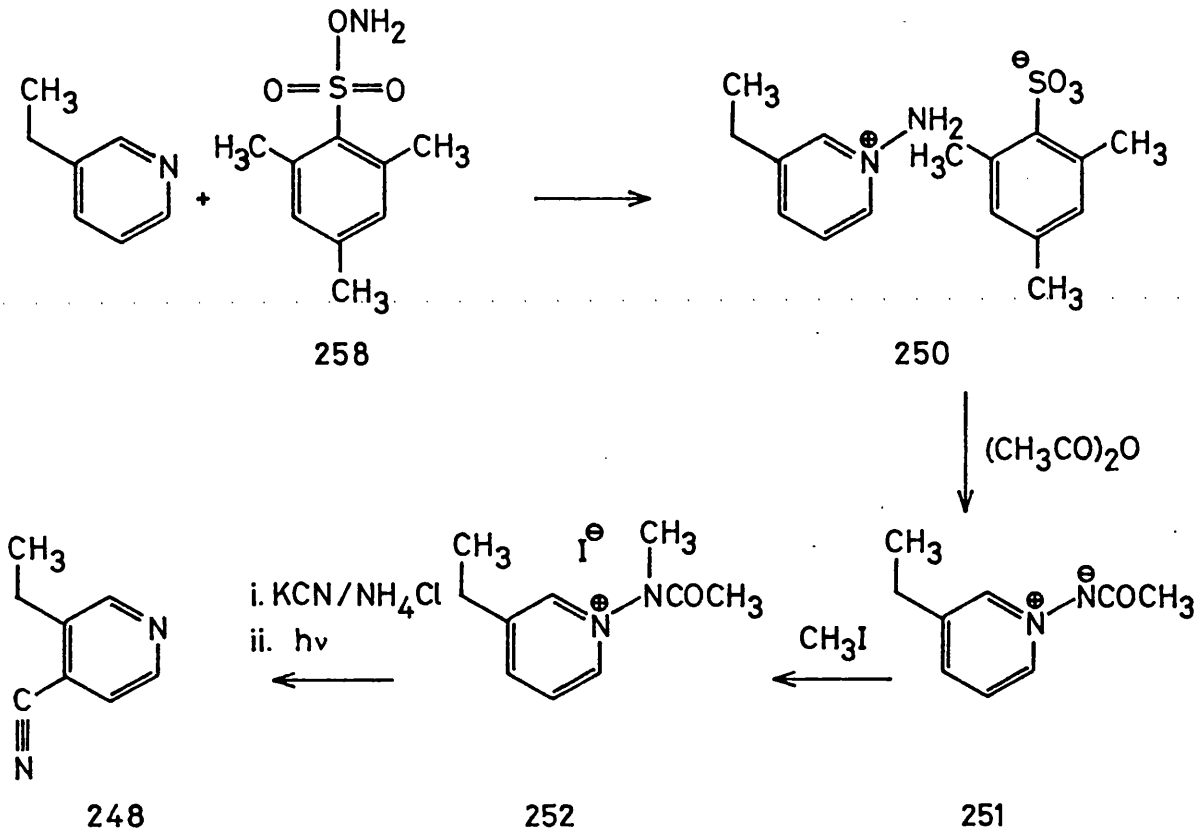
The preparation of 4-acetyl-3-ethylpyridine (237) was undertaken using 3-ethylpyridine as the starting material *via* the so-called "MSH route" developed in this laboratory, which gave 4-cyano-3-ethylpyridine (248) as an oil. Treatment with methyllithium afforded the imine (249) which was immediately hydrolysed to the acetyl derivative with refluxing 30% acetic acid. Unfortunately all of the intermediate compounds in this reaction

sequence were liquids with their inherent handling problems which resulted in a yield over seven steps of a mere 18%.

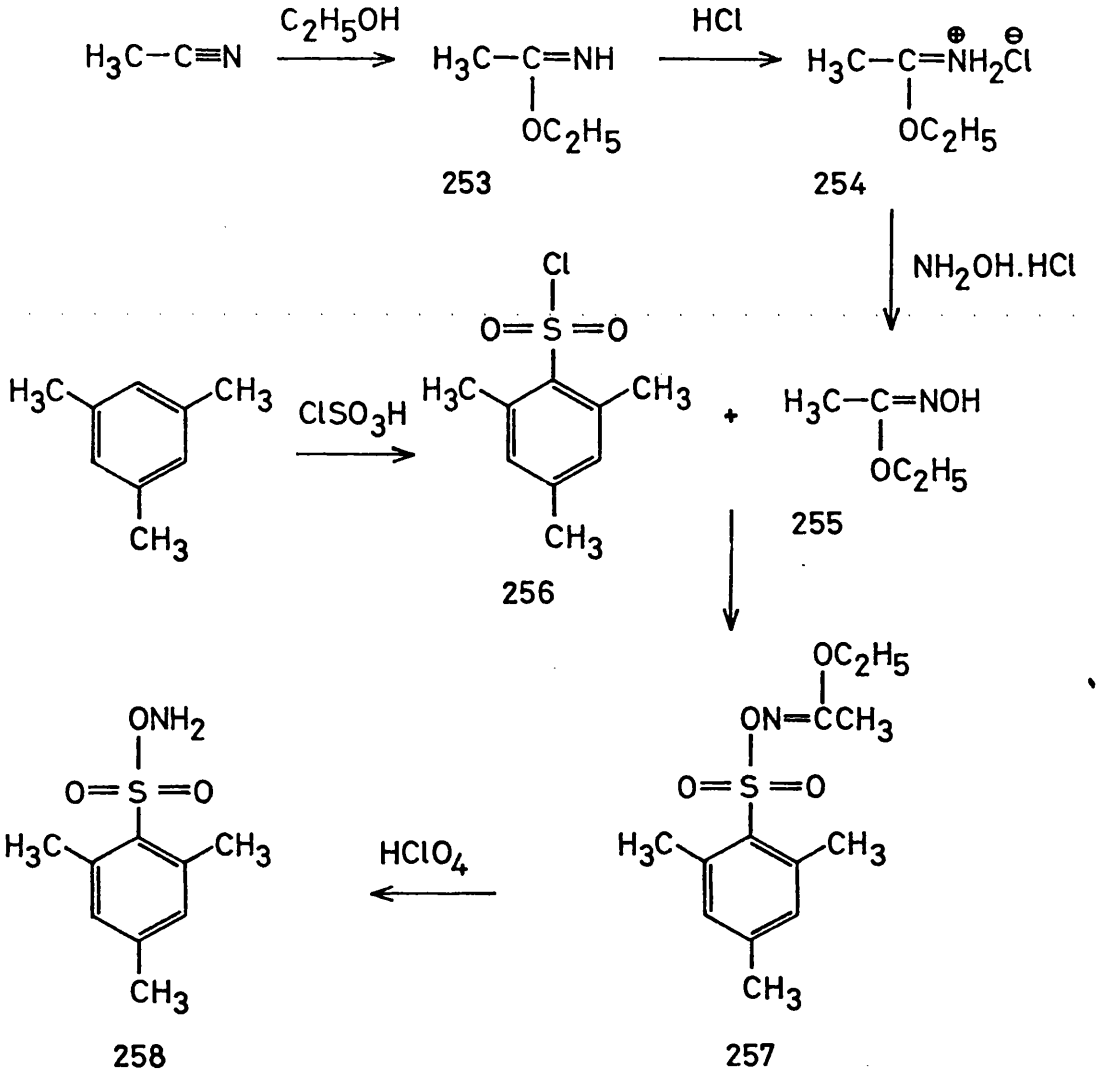


248 249 237

Activation of the 3-ethylpyridine nucleus towards attack by cyanide ion was achieved by formation of a pyridium salt, in this case the amino-mesitylenesulphonate (250). Treatment of this compound with acetic anhydride gave an unstable acetylimide intermediate (251) which was immediately quarterised to the methiodide compound (252) by reaction with excess iodomethane at reflux. In this form, not only was the pyridine nucleus activated in the desired manner, but in addition the 2- and 6- positions were effectively blocked to approaching nucleophiles by the bulky N-methylacetamide function attached to the heteroatom. Thus the 4- position was the only favourable site for nucleophilic attack to occur.

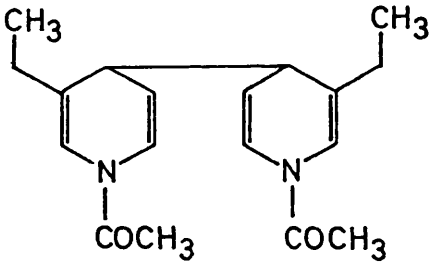


The aminating agent itself was prepared according to the following reaction sequence: Mesitylenesulphonyl chloride (256) was prepared by the action of chlorosulphonic acid on mesitylene, whilst 1-ethoxy-1-oximidoethane (255) was obtained from acetonitrile. The nitrile was ethoxylated in dry ether using absolute alcohol and the salt (254) was formed by treatment with dry hydrogen chloride gas. Treatment of this compound with potassium carbonate and hydroxylamine hydrochloride gave the oximidoethane (255) as a colourless oil which was condensed with mesitylenesulphonyl chloride to give O-(mesitylenesulphonyl) acetohydroxamate (257). Since mesitylenesulphonyl hydroxylamine (258) is an unstable compound and may decompose violently the material was stored as this precursor and hydrolysis to the aminating agent was carried out as and when required, by the action of 60% perchloric acid.

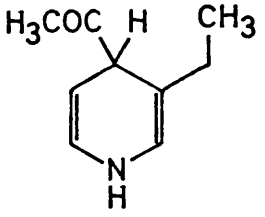


In order to ascertain whether the synthesis of 4-acetyl-3-ethyl-pyridine could be improved, alternative preparative methods were sought. The procedure developed by Wibaut and Arens[122,123] seemed particularly attractive since the number of steps involved is very small. Accordingly, 3-ethylpyridine was treated with acetic anhydride in the presence of zinc powder in a reaction intended to give N,N^{1}-diacetyl-3,3^{1}-diethyltetrahydro-4,4^{1}-dipyridyl (259). After removal of the residual zinc powder and zinc acetate by filtration, a yellow solution was obtained from which the excess acetic anhydride was

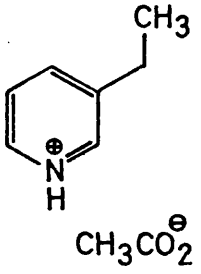
evaporated under reduced pressure at a moderate temperature to leave a yellow oil. This was distilled under vacuum as a single fraction boiling at 40° at a pressure of 0.15 mm Hg. However, the ^1H nuclear magnetic resonance spectrum of this compound was difficult to rationalise in terms of structure (260), being comprised of a one-proton singlet at δ14.2; a two-proton double doublet (J_1=2HZ and J_2 = 5HZ) at δ8.5; a one-proton pair of triplets (J_3 = 2HZ and J_4=8HZ) at δ7.63; a one-proton double doublet (J_5=5HZ and J_6=8HZ) at δ7.27; a two-proton quadruplet (J_7=7HZ) at δ2.66; a three-proton singlet at δ2.08 and a three-proton triplet (J_8=7HZ) at δ1.24 ppm. Although the compound contains thirteen protons, the aromatic signals are not consistent with the dihydropyridine structure (260) illustrated below:



259



260



261

In fact the aromatic pattern of signals is closely related to 3-ethylpyridine itself, for which the high field double doublet arises from two ortho - couplings of the C-5 proton with the C-4 and C-6 protons. The resonance due to the C-4 proton appears as a more complex signal because here there are two meta - couplings with the protons at C-1 and C-6, as well as the ortho - coupling with the C-5 proton. Protons at C-1 and C-6 both appear at low field and probably experience similar coupling interactions with the C-4 proton. Thus the signal for the proton at C-6 appears as a double doublet which masks the doublet due to the C-1 proton. It therefore became apparent

that the anomalous spectrum was due to an association complex between 3-ethylpyridine and acetic acid, represented here as structure (261).

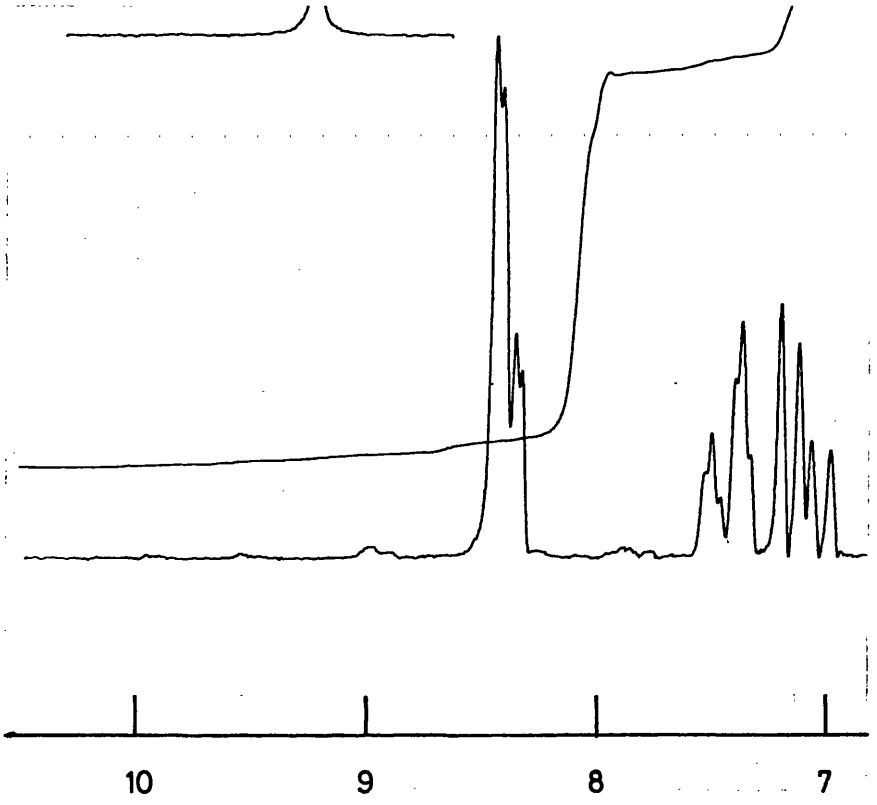
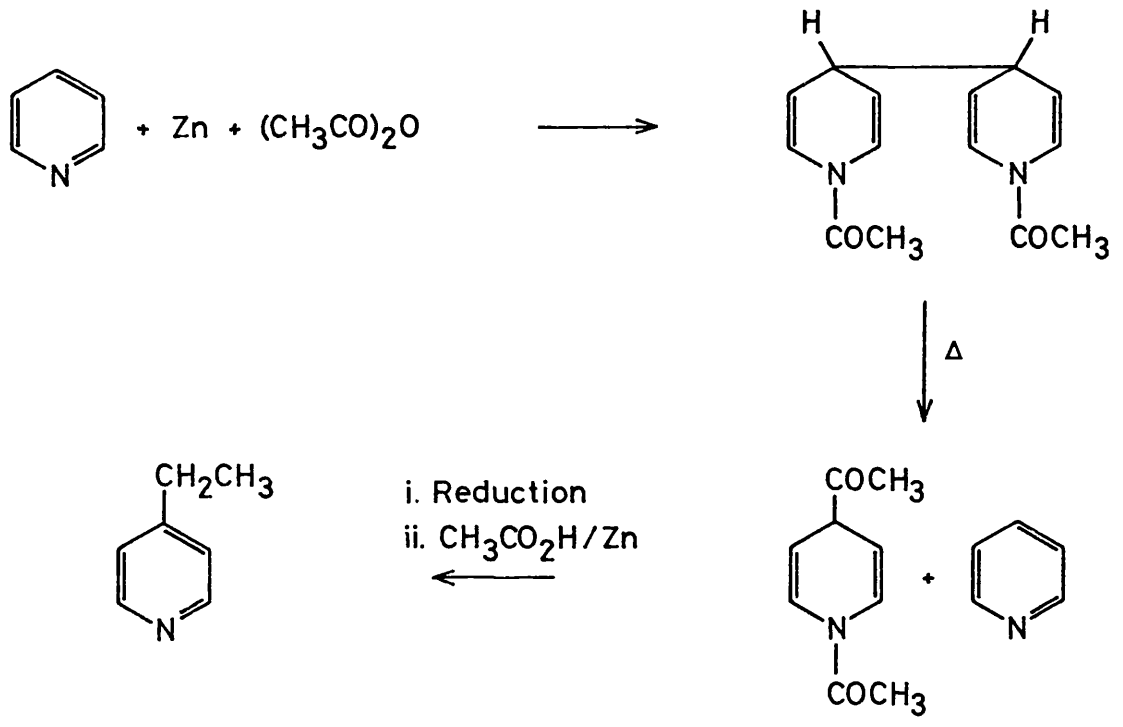


Figure 4. Aromatic proton resonances for the 3-ethylpyridine Wibaut-Arens reaction product

It was not surprising, then, that subsequent treatment of the oil which was aimed at promoting dissociation to 4-acetyl-3-ethylpyridine and starting material, resulted only in the isolation of unchanged 3-ethylpyridine.

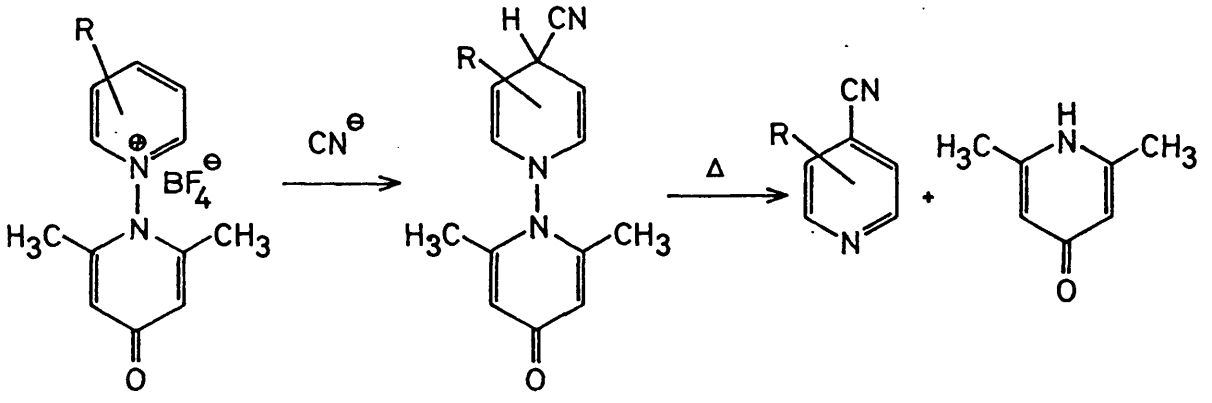
Previously at Bath, the Wibaut-Arens reductive acylation procedure has been employed with only limited success. The reaction has seldom been reproducible, and it was the need for an effective means of synthesising 4-substituted pyridines which prompted the development of the MSH route. The reasons for this apparent failure are not obvious, although it might be argued that 3-substituted pyridines are unsuitable for this type of reaction because of steric effects. Nevertheless, Woodward's

group employed this reaction for an even more highly hindered 3-substituted pyridine substrate in their original ellipticine synthesis[20]. (See Scheme 1, page 15). Certainly, in the preparation of 4-ethylpyridine from pyridine itself, as described by Wilbert, Reich and Tenenbaum[125], the reaction works well and conditions have been optimised so that the yield of the alkylated product exceeds 75%. These workers also found that the reaction was best carried out at elevated temperatures since this gave better control over the violent exothermic reactions which occurred during certain stages of the original procedure:



It would have been valuable to devote a good deal of time to a more extensive study of the Wibaut-Arens reaction in order to establish its scope and limitations in the context of ellipticine syntheses. However, the preparation of 4-acetyl-3-ethylpyridine was a more immediate objective and fortunately a second alternative became available which in some respects parallels the use of 1-(N-

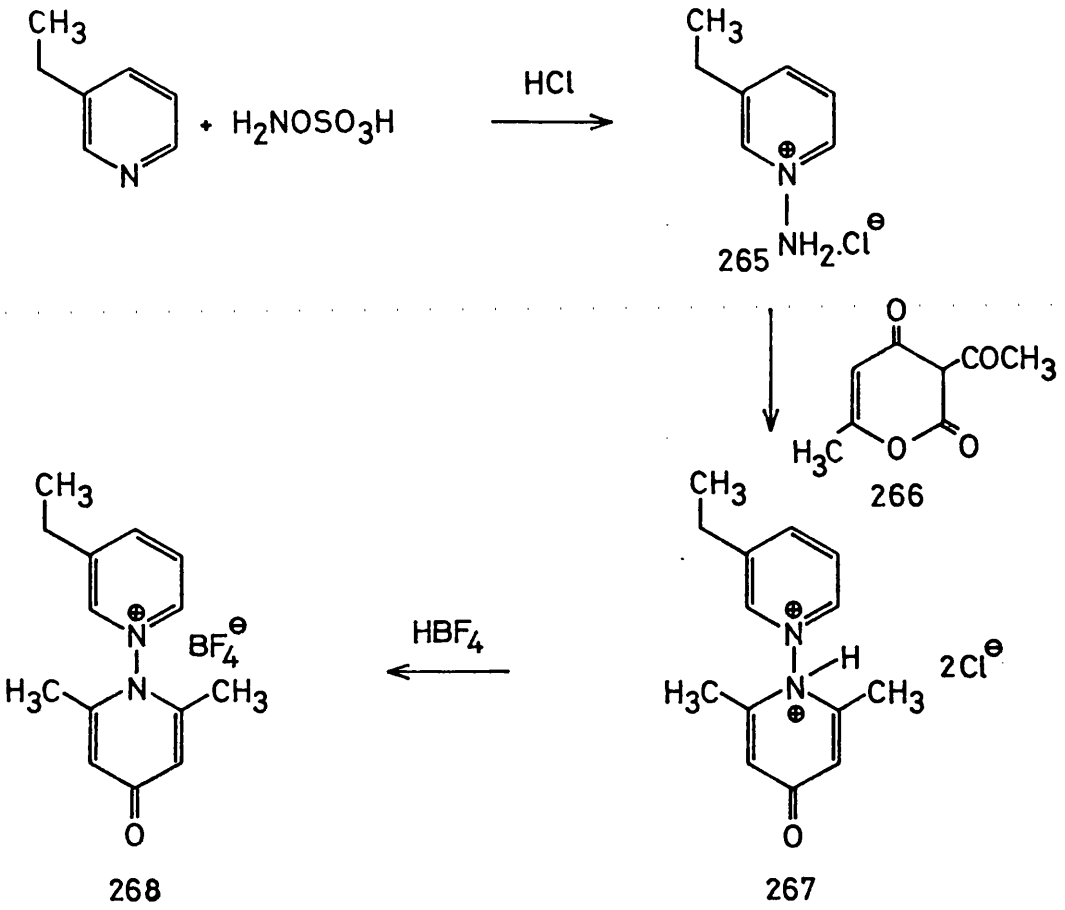
acylalkylamino)pyridinium salts. Sammes and Katritzky[103,126] report that pyridiniopyridones of the type (262) react with cyanide ion in aqueous solution, giving essentially exclusive attack at the 4- position of the pyridinium ring. High yields of the intermediate dihydropyridines (263) were obtained which could be converted almost quantitatively into 4- cyanopyridines (264) by warming to 60^{o} at a pressure of 1mm Hg.



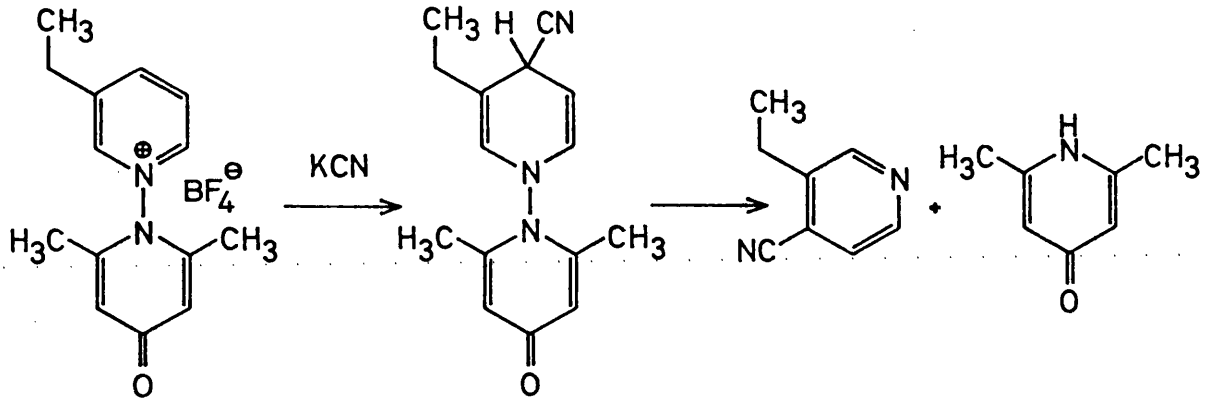
262 263 264

It was decided, therefore, to adopt this procedure for the preparation of 4- cyano-3-ethylpyridine (248) as an alternative to the scheme illustrated on page 132.

Hydroxylamine-O-sulphonic acid, formed by the action of chlorosulphonic acid upon hydroxylamine hydrochloride, was reacted with 3-ethylpyridine according to the method of Meuwsen and Gösl[127] to give 1-amino-3-ethylpyridinium chloride (265) as a hygroscopic green solid. This compound was then treated with dehydroacetic acid (266) in boiling hydrochloric acid to give 2,6-dimethyl-1-(3-ethyl) pyridinio-4-pyridone as its chloride hydrochloride (267). A saturated solution of the double salt in absolute ethanol was added to tetrafluoroboric acid and stirred, whereupon a precipitate of the desired pyridone (268) was obtained in a yield of 23% from the starting pyridine.



Reaction of the pyridone (268) with potassium cyanide in aqueous solution proceeded smoothly and in excellent yield. There were no solubility problems of the kind which had previously lessened the efficiency of the reaction between cyanide ion and the methiodide salt (252). Apparently, from the evidence of its ^1H nuclear magnetic resonance spectrum, disproportionation of the dihydropyridrine species (269) occurred spontaneously to give a mixture of 4-cyano-3-ethylpyridine (248) and 2,6-dimethyl-4-pyridone:



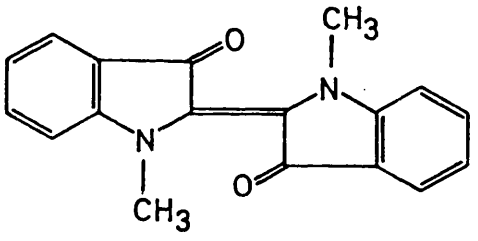
268 269 248

Separation of these two compounds was surprisingly difficult, and eventually the pyridine nitrile was obtained in pure form by fractional distillation under reduced pressure. By this method of preparation, the overall yield of the cyanide compound was a disappointing 15%.

Nevertheless, this procedure has certain advantages over the MSH route, since fewer steps are involved and also because readily available materials are used throughout. The major drawback is without doubt the poor yield, especially when it is considered that each intermediate compound is isolated as a solid. As yet this procedure has not been applied to more complex pyridines such as 3-[1-(3-pyridyl)ethyl]indole (29) or derivatives thereof, since it is known that the MSH route gives very good results for this class of compounds.

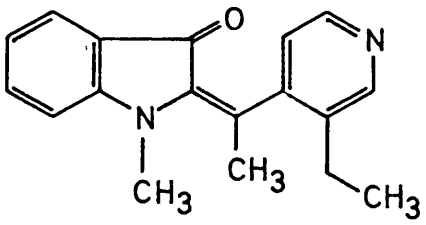
When sufficient quantities had been prepared, 1-methylindol-3-ol acetate (236) and 4-acetyl-3-ethyl-pyridine (237) were combined in aqueous methanol solution containing potassium hydroxide in a nitrogen-purged flask which was then tightly stoppered and left to

stand for three days. After this time the mixture was filtered and the solvents removed under reduced pressure to leave a dark amorphous solid. Analysis of this material by TLC showed that it comprised two major components, one of which was deep blue in colour and remained virtually stationary on the plate. It was speculated that this was the undesired indigotin by-product (270) resulting from the action of base and atmospheric oxygen on hitherto unreacted indoxyl:

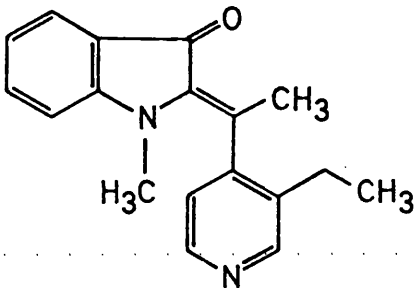


270

Extensive chromatographic work was carried out in an effort to obtain the major component in pure form, but all attempts were thwarted. It was thought that the reason for this might be that the indoxylidene would be expected to exist as spatial isomers (238a) and (238b). Since it was desired to have a single, fully-characterised reactant for the attempted ring closure reaction in order to determine its outcome, this material was treated with sodium borohydride and attempts were made to purify the resultant product. Once again, however, column chromatography failed to yield a single component which could then be recrystallised. When a second reaction between 4-cyano-3-ethylpyridine and 1-methylindol-3-ol acetate gave similar results, it became evident that this procedure would not prove as straightforward as had been anticipated. Therefore it was decided to shelve this approach until such time as new techniques became available which might overcome the difficulties encountered here.



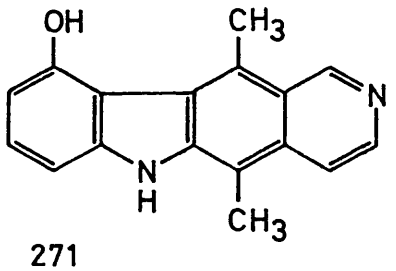
238a



238b

Synthetic efforts towards 10-hydroxyellipticine (271):

The importance of hydroxylated derivatives of ellipticine is now widely acknowledged. 9-Hydroxyellipticine in particular has been the subject of numerous publications[112,128-130], perhaps because it is one of the major mammalian metabolites of ellipticine itself and has been shown by Le Pecq et al.[16] to be a potent anti-tumour agent. Chien and Rosazza[130] report that microbial transformation of ellipticine by Aspergillus alliaceus gives not only the 9-hydroxy compound, but also smaller quantities of 8-hydroxyellipticine, a hitherto unknown compound which has been the target of a recent synthesis by Dolman and Sainsbury[131] at Bath. The availability of a natural source of this compound was valuable in confirming the regiospecificity of the synthetic route employed by these workers.



271

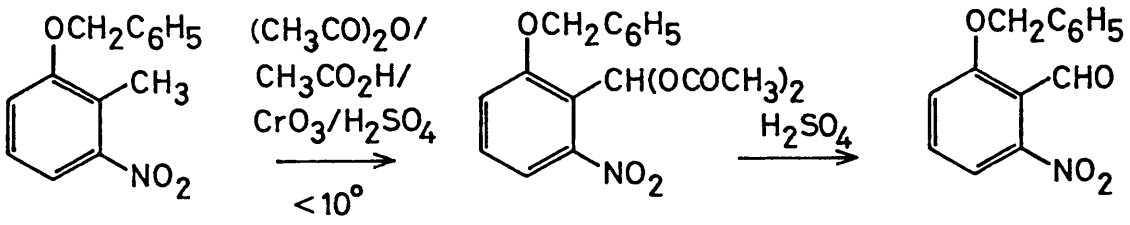
To date, no synthesis of 10-hydroxyellipticine (271) has been recorded, and since the other phenolic derivatives have been the subject of evaluation studies against experimental tumour systems, it was felt that preparation of this compound would be a valuable exercise.

Accordingly, preparation of a sufficient quantity of 4-hydroxyindole (283) as its methoxylated derivative (282) became the initial objective, since conversion of indole systems into the

desired tetracycle was by now a familiar technique. Using 2,6-dinitrotoluene as the starting material, treatment with sodium polysulphide effected conversion to 2-methyl-3-nitroaniline (272) in excellent yield. This material, in the presence of acid, was then treated with sodium nitrite in an ice bath, and the resultant diazonium salt was then refluxed in sulphuric acid to give the phenol (273) as an orange solid.

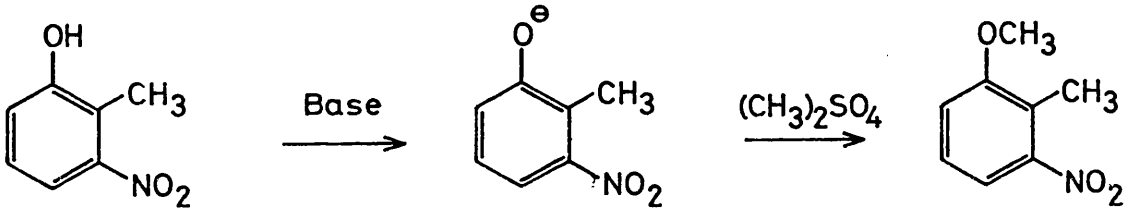
The phenolic group was originally protected by converting it to the benzyloxy function through the action of benzyl chloride in the presence of base. However, fears that the benzyloxy compound (274) would be too severely sterically hindered for efficient reaction in subsequent steps proved to be well-founded when attempts were made to oxidise the methyl side chain.

At first an attempt was made to prepare 2-benzyloxy-6-nitrobenzaldehyde (276) via the benzaldiacetate (275) by treating the parent compound with acetic acid, acetic anhydride and chromium trioxide in the presence of sulphuric acid at depressed temperature. Unfortunately the expected product was not obtained in this reaction, an intractible oil being the result. It was therefore decided to try an alternative protecting group for the phenolic compound in the hope that steric hindrance might be minimised about the site of the methyl side chain.



274 275 276

In their preparation of 2-methoxy-6-nitrotoluene (278), Roberts, Wiles and Kent[132] used dimethyl sulphate in the presence of anhydrous potassium carbonate to alkylate the phenol. No yield was recorded for this reaction, however, and it was felt that the phase-transfer catalytic procedure developed by McKillop, Fiaud and Hug[133] might be useful here since the phenolic substrate was slightly hindered. Furthermore, reaction in aqueous solution proceeds via the phenoxide ion (277), which is destabilised through having a nitro group in the meta-position to the hydroxyl function.



273 277 278

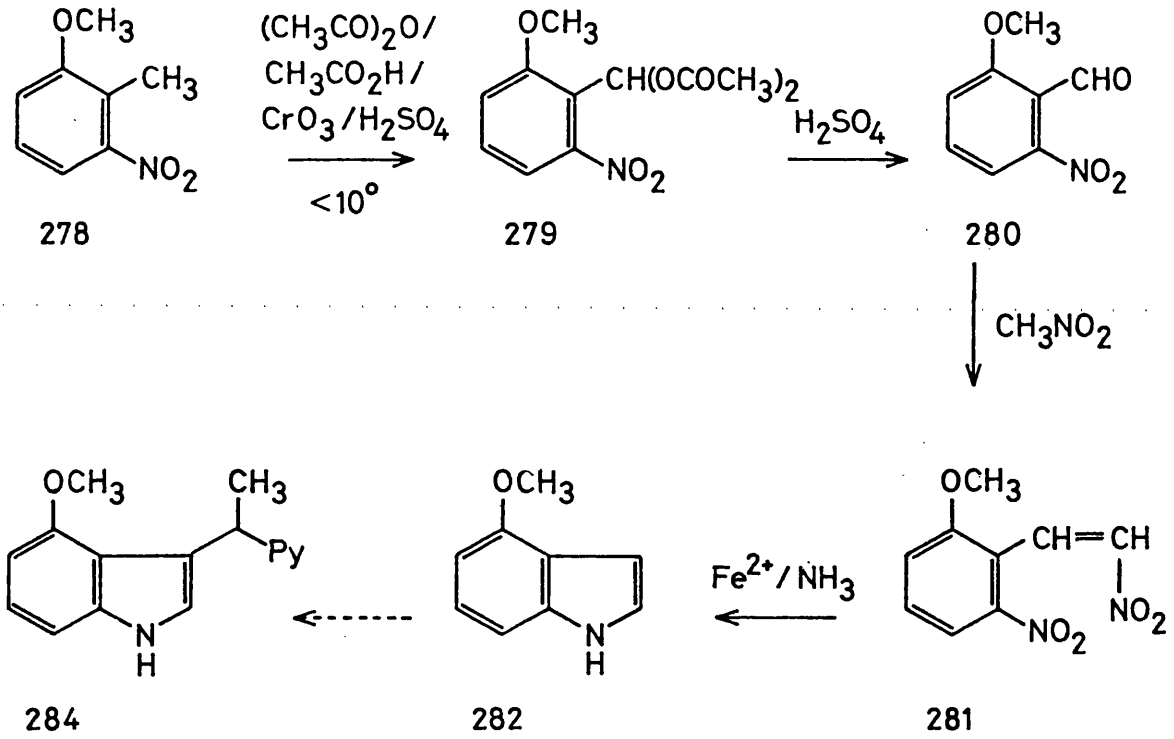
The phase-transfer technique takes advantage of the limited population of phenoxide ions, which are converted into the corresponding quaternary ammonium phenoxide in the aqueous phase. This latter salt has a discrete solubility in the organic phase and consequently transport of the phenoxide ion into the organic layer is followed by rapid irreversible alkylation and formation of the methyl ether (278). This product remains largely dissolved in the organic layer and is thus effectively removed from the reaction mixture, thereby encouraging the equilibrium to proceed in the desired direction.

By using benzyl tri-n-butylammonium bromide[134] as the phase-transfer catalyst here, it was possible to obtain 2-methoxy-6-nitrotoluene in better than 90% yield from the phenol (273) in

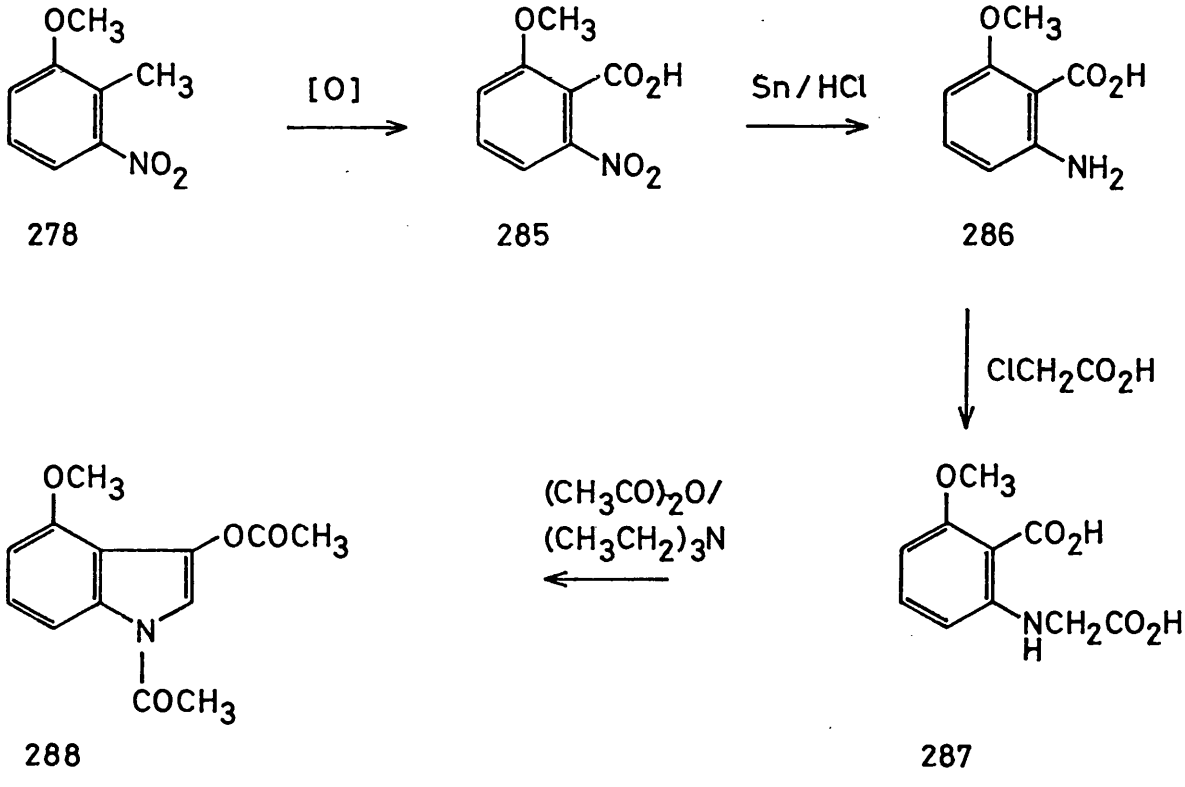
what might otherwise have been a reaction of somewhat modest yield

Before subjecting the methoxy compound to the rigorous oxidation conditions previously described, a model reaction was carried out using 2,6-dinitrotoluene as the test substance. At the depressed temperature this procedure returned unchanged starting material so the reaction was repeated, allowing the temperature of the reaction mixture to rise to 20^{0} after the addition of sulphuric acid. Normally, this would have been expected to result in complete oxidation of the side chain to a carboxylic acid group[135], but once again 2,6-dinitrotoluene was isolated as the sole product. These observations indicated that steric hindrance about the alkyl side chain was really quite acute and that perhaps an alternative method should be sought. Nevertheless, when the methoxy compound was treated in this manner, a modest yield of 2-methoxy-6-nitrobenzaldiacetate (279) was obtained and this material was then hydrolysed in refluxing sulphuric acid to give 2-methoxy-6-nitrobenzaldehyde (280) in an overall yield of 8%. Under these circumstances it became apparent that preparation of 4-methoxyindole *via* the scheme illustrated over was not really a viable proposition.

Moreover, a large quantity of the indole (282) would have to be prepared in order to ensure that a sufficient amount of 4-methoxy-3-[1-(3-pyridyl) ethyl] indole (284) could be obtained through reaction of the Grignard derivative with 3-(1-chlorethyl) pyridine (73). Thus it was felt that preparation of the corresponding indoxyl (288) *via* the scheme illustrated below might ultimately prove more successful, since this required that the methyl side chain should be oxidised to a carboxylic acid group, a transformation which could be achieved in a single step using a powerful oxidant.



278 279 280 281 282 284

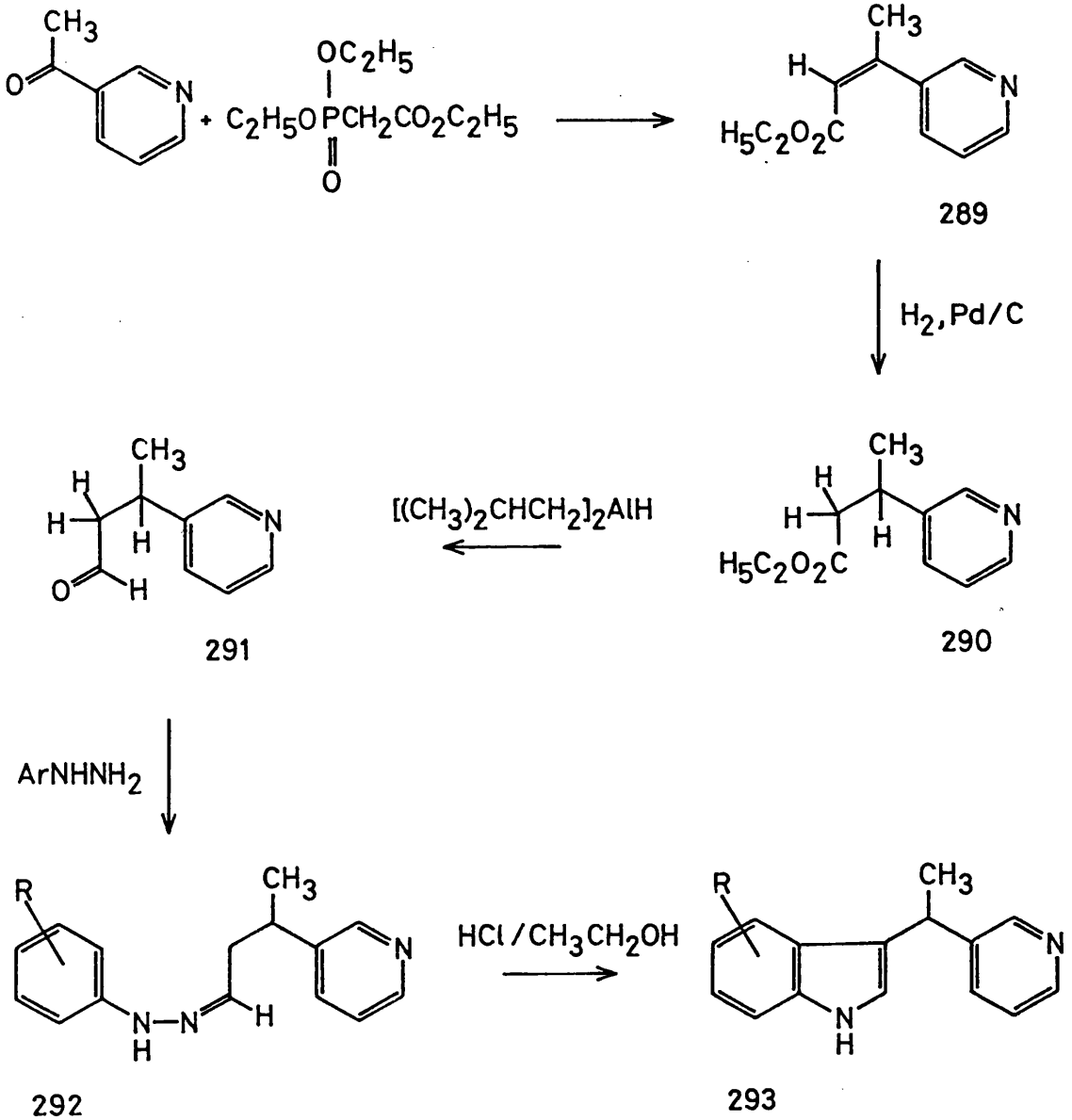


278 285 286 287 288

Accordingly, 2-methoxy-6-nitrotoluene (278) was mixed with potassium dichromate as sulphuric acid was added cautiously. After completion of the addition, the mixture was brought slowly to the boil and refluxed steadily for about half an hour. On work-up, however, analysis by TLC indicated that only unreacted starting material was present, even when the reaction was repeated with a prolonged period of reflux during an entire weekend. Consequently an alternative method was sought: Treatment of the methoxy compound with potassium permanganate was at least partially successful in effecting conversion to the acid (285). However, 47% of the starting material was returned unchanged and thus the isolated yield of 2-methoxy-6-nitrobenzoic acid was only 27%. Whilst this yield could be enhanced when the unconsumed starting material was recycled, a large number of repeat reactions was necessary to prepare sufficient acid for conversion into the anthranilic acid derivative (286). Regrettably, there was insufficient time to complete this process and the work was suspended at the benzoic acid stage.

Recently a new synthesis of 3-[1-(3-pyridyl)ethyl] indole (293) has been devised at Bath[136] using the pyridine aldehyde (291). This key intermediate is obtained from 3-acetylpyridine by a Wittig reaction with triethylphosphonoacetate to give the unsaturated ester (289) which is reduced to the saturated compound (290) by catalytic hydrogenation. Reduction of the ester function is achieved in excellent yield using diisobutylaluminium hydride to give the desired aldehyde (291). Reaction of this compound with a suitably substituted arylhydrazine gives a hydrazone (292) which may be cyclised to the indole using Fischer conditions. It has been observed that 3-substituted arylhydrazines give rise to 4-

and 6- substituted indoles in the approximate ratio of 3:1, and it is hoped that in the near future this technique might be used to prepare 4-methoxy-3-[1-(3-pyridyl)ethyl] indole (284), as well as the corresponding 6-methoxy compound, in sufficient quantity to facilitate synthesis of 10-methoxy- and 10-hydroxyellipticines.



EXPERIMENTAL

EXPERIMENTAL

Preparation of 3-(1-hydroxyethyl) pyridine (145)

To 3-acetylpyridine (275g, 250cm^3) dissolved in ethanol (1dm^3), was added sodium borohydride in small portions over a period of thirty minutes with stirring. The temperature was maintained below 30^o by means of an ice bath, and the reaction was judged to be complete by the observation of a constant ultra violet spectrum. Distilled water (100cm^3) was then added and the ethanol was removed by evaporation under reduced pressure. The resulting yellow slurry was diluted with distilled water (750cm^3) and extracted with chloroform (5 x 200cm^3). The organic extracts were combined and dried over magnesium sulphate. After filtration the solvent was removed under reduced pressure to give a pale yellow oil which was distilled under vacuum (90^o, 0.3mmHg) to yield the product as a colourless oily liquid (240g, 87%).

^{1}H N.M.R. $\delta(CDCl_3)$ 8.51(1H,d,J=2HZ,H-2),
8.41 (1H,dd,J=2HZ and J=5HZ,H-6),
7.81 (1H,dt,J=1HZ and J=8HZ,H-4),
7.29 (1H,dd,J=5HZ and J=8HZ,H-5),
5.40 (1H,bs,OH),
4.90 (1H,q,J=6HZ,$CHCH_3$),
and 1.47 (3H,d,J=6HZ,$CHCH_3$)ppm,

I.R. νmax 3250b(O-H) and 1410(O-H)cm^{-1},

U.V. λmax 255,260 and 266nm,

M.S. m/e (rel.int.%) 123(M^+,45), 108(100) and 80(39),

b.p. 90° (0.3mmHg).

Preparation of 3-(1-chloroethyl) pyridine (73):

3-(1-hydroxyethyl) pyridine (20g) was dissolved in dry toluene ($50cm^3$) and cooled in an ice bath. Freshly-distilled thionyl chloride ($120cm^3$) was added dropwise with stirring to give a cloudy suspension. The mixture was evaporated under reduced pressure to give a yellow jelly which was dissolved in iced water ($50cm^3$) and washed with ether ($3x20cm^3$). The aqueous suspension was made basic with solid sodium hydrogen carbonate and extracted with ether ($3x100cm^3$). The combined organic extracts were washed with water, dried over sodium sulphate, filtered and the solvent evaporated under reduced pressure to give the chlorinated compound as an unstable pale yellow liquid (22g, 96%).

^1H N.M.R. δ (d^6 DMSO) 8.70 (1H,d,J=2HZ,H-2),
8.55 (1H,dd,J=2HZ and J=5HZ,H-6),
7.92 (1H,dt,J=2HZ and J=9HZ,H-4),
7.42 (1H,dd,J=5HZ and J=9HZ,H-5),
5.39 (1H,q,J=7HZ,$C\underline{H}CH_3$),
and 1.82 (3H,d,J=7HZ,$CHC\underline{H}_3$)ppm

I.R. νmax 1580 (Ar)cm^{-1}.

Preparation of 3-[1-(3-pyridyl)ethyl]indole (29):

a. From indolylmagnesium bromide and 3-(1-chloroethyl)pyridine(73):

Dry magnesium turnings (3.4g, 0.14 mole) were placed in a $100cm^3$ round-bottom, three neck flask under a stream of nitrogen, and then anhydrous ether ($20cm^3$) was added. Bromoethane ($10.8cm^3$,15.8g, 0.145 mole) was dissolved in anhydrous ether ($25cm^3$) and a few drops of this solution were added to the contents of the flask to initiate reaction. When a slight cloudiness was discernible, stirring was commenced and the remainder of the bromoethane solution was added at a rate which maintained the ether at reflux temperature. After

forty-five minutes the magnesium had dissolved to leave a cloudy grey suspension. The ethereal solution of ethylmagnesium bromide was cooled in an ice-salt bath and a solution of indole (16.6g, 0.142 mole) in anhydrous ether ($50cm^3$) was added dropwise over a period of half an hour. The resulting suspension was allowed to warm to room temperature and was stirred for a further hour.

The reaction mixture was then cooled in an ice-salt bath and 3-(1-cholorethyl) pyridine was added in a single portion with stirring. Stirring was maintained for a further forty-eight hours while the contents of the flask warmed slowly to room temperature, during which time a colour change from pale grey to dark brown was observed. The dark product was cooled to 0° and extracted with hydrochloric acid ($5x50cm^3$,2M). The combined extracts were washed with ether and basified by the cautious addition of concentrated ammonia solution when a pale yellow flocculate separated. The mixture was extracted with chloroform ($3x100cm^3$) and the combined extracts were dried over magnesium sulphate, filtered and the solvent evaporated under reduced pressure to give an oil. The oil was triturated with ether to give a pale straw-coloured solid which was collected at the pump and washed with ether. Recrystallisation from ethanol afforded the product as white prisms (3.3g,21%).

b. <u>From indolylmagnesium bromide and 3-(1-chloroethyl)pyridine; optimised conditions:</u>

A small but significant improvement in the yield of this reaction was achieved by using a greater dilution of the reagents in anhydrous ether so that after the addition of indole a total volume of $200cm^3$ of ether had been used. Partial homogenisation of the indolylmagnesium bromide suspension was brought about by adding

3-(1-chloroethyl)pyridine dropwise as a solution in dichloromethane ($10cm^3$).

Finally, in the extraction procedure, the product was exhaustively extracted with hot dilute hydrochloric acid (ca.$5x200cm^3$) until very little of the dark brown gum remained. In this way it was possible to isolate the product in 24% yield.

^{13}C N.M.R. $\delta(d^6$-DMSO)

2'	3'	3a	4'	5'	6'	7'	7a	$\underline{C}HCH_3$
126.2d	118.3s	123.2s	121.0d	121.8d	118.6d	111.4d	141.5s	33.9d

$CH\underline{C}H_3$	2	3	4	5	6	
21.9q	148.8d	131.4s	136.7d	121.0d	147.0d	ppm,

^1H N.M.R. δ(CDCl) 9.58 (1H,bs,N$\underline{H}$),
8.66 (1H,d,$\underline{J}$=2HZ,H-2),
8.45 (1H,dd,$\underline{J}$=2HZ and $\underline{J}$=6HZ,H-6),
7.70-6.95 (7H,complex,H-4,H-5,H-2',H-4',H-5',H-6' and H-7'),
4.50 (1H,q,$\underline{J}$=8HZ, C$\underline{H}CH_3$),
and 1.82 (3H,d,$\underline{J}$=8HZ,$CHC\underline{H}_3$)ppm,

I.R. νmax 3150 (N-H),1590 and 1580 (Ar)cm^{-1},

U.V. λmax 229,270,280 and 291 nm,

M.S. m/e (rel.int.%) 222 (M^+,60), 207(100) and 144(14),

M.P. 173°(lit.[41,42], 173-174°).

c. From indolylmagnesium bromide and 3-(1-chloroethyl) pyridine; investigation of reaction time:

Using the above procedure, a series of four experiments was conducted in which the stirring time was varied after the addition of 3-(1-chloroethyl) pyridine was complete. Reaction mixtures were then worked up in the customary way. The stirring times and yields of isolated product are tabulated below:

STIRRING TIME/h	YIELD/g (%)
2	0.5 (3)
24	1.7 (11)
48	3.8 (24.5)
170	3.7 (24)

d. From indol -3-yl-3-pyridyl methanone (111) and methyllithium:

Methyllithium solution in hexane (24.2cm, 1.24M, 0.03 mole) was placed in a 100cm^3 round-bottom, three-neck flask under a stream of dry nitrogen, then indol-3-yl-3-pyridyl methanone (111) (2.22g, 0.01 mole) suspended in dry toluene (50cm^3) was added dropwise to the stirred solution over a period of about twenty minutes. The temperature was maintained below -5° using an ice-salt bath and stirring was continued overnight whilst the reaction mixture gradually warmed to room temperature. The excess organometallic reagent was then decomposed by the cautious addition of a solution of ammonium chloride (10g) in water (40cm^3). The organic layer was separated, washed with brine, dried over magnesium sulphate, filtered and the solvent evaporated under reduced pressure to give a yellow solid.

This material showed two major spots on TLC, neither of which corresponded to the starting ketone (111). Attempts to recrystallise the product or separate the components chromatographically were unsuccessful and consequently no satisfactory ^{1}H NMR data were recorded.

I.R. ν max 3150 (N-H), and 1620 (conjugated C=C) cm^{-1},

U.V. λmax 270,290 and 315nm,

M.S. m/e (rel.int.%) 220 (M^+,5), 92(44) 91(44) and 88 (100).

The product from the above reaction was treated with a variety of reducing agents in an attempt to effect conversion to 3-[1-(3-pyridyl) ethyl] indole:

i. Reaction with sodium borohydride:

A portion of the yellow solid (0.5g) was dissolved in ethanol and the stirred solution was treated with portions of sodium borohydride until a constant ultra violet spectrum was observed. The excess borohydride was decomposed by the addition of acetone, resulting in a yellow slurry, The solvent mixture was evaporated under reduced pressure and the orange-coloured residue was partitioned between chloroform ($50cm^3$) and water ($50cm^3$). The organic phase was separated, dried over magnesium sulphate, filtered and the solvent removed under reduced pressure to give an orange solid. Analysis of this material by TLC showed it to be a multi-component mixture. The main peaks in the mass spectrum are recorded below:
M.S. m/e (rel.int.%) 222(77), 208(100), 207(98) and 144 (38).

ii. Reaction with lithium aluminium hydride:

The yellow solid (0.5g) was dissolved in freshly distilled anhydrous tetrahydrofuran ($50cm^3$) and was treated with lithium aluminium hydride at room temperature. After stirring for several hours the excess reducing agent was decomposed by the cautious addition of potassium sodium tartrate solution and the mixture was filtered at the pump to remove the precipitated salts. The precipitate was thoroughly washed with tetrahydrofuran. The organic solvents were removed by evaporation under reduced pressure to leave a suspension in water which was extracted into chloroform ($2x20cm^3$). The organic extracts were combined, dried over magnesium sulphate, filtered and the solvent removed under reduced pressure to give a buff-coloured

solid. The isolated material proved to be almost identical with the starting compound on TLC and there was no evidence in the mass spectrum to suggest that the desired 3-[1-(3-pyridyl) ethyl] indole had been formed.

iii. Reaction with hydrogen on platinum oxide:

A portion of the yellow solid (0.5g) was dissolved in ethanol ($100cm^3$) and hydrogenated under a pressure of 120 p.s.i. of hydrogen in the presence of platinum oxide for about twenty hours. The reaction mixture was filtered through Kieselguhr and the filtrate evaporated to a gum under reduced pressure. The gum was dissolved in a little chloroform and eluted through a short column of silica gel. The combined fractions were dried over magnesium sulphate, filtered and the solvent removed under reduced pressure to give a buff-coloured solid. This compound was found to be the same as the starting material on examination by TLC.

e. From indol-3-yl-3-pyridyl methanol (112) and methyllithium:

A solution of methyllithium/lithium bromide complex in diethyl ether ($12cm^3$, 1.3M, 0.017 mole) was placed in a small round-bottom flask carrying a steady stream of dry nitrogen and the apparatus was immersed in an ice-salt bath. A solution of indol-3-yl-3-pyridyl methanol (112) (1.12g, 0.005 mole) in anhydrous tetrahydrofuran ($20cm^3$) was added dropwise to the stirred contents of the flask and the mixture was allowed to warm to room temperature slowly overnight. The excess organometallic reagent was then decomposed by the cautious addition of a solution of ammonium chloride (10g) in water ($40cm^3$). The organic layer was separated, dried over magnesium sulphate, filtered and the solvent removed under reduced pressure to give an intractible gum. Analysis of this material by TLC showed it to be

very complex in nature and no further investigation was carried out.

f. From isatin and 3-(1-chloroethyl) pyridine (73):

Dry magnesium turnings (0.4g) were placed in a 100cm^3 round-bottom, three-neck flask and a solution of 3-(1-chloroethyl) pyridine (2g) in dry ether (40cm^3) was allowed to run into the flask until the magnesium turnings were just covered. The reactants were then left to stand but the reaction did not commence. Stirring failed to initiate the reaction, as did the addition of a crystal of iodine and warming the solution to reflux temperature in a water bath.

In a second attempt to react 3-(1-chloroethyl) pyridine with isatin, the lithium organometallic reagent was prepared in the following manner: Lithium (0.4g, 4 mole equivalents) was cut into small pieces and allowed to drop directly into a 100cm^3 round-bottom, three-neck flask containing rigorously dried tetrahydrofuran (20cm^3). In this way the bright lustre was maintained on the surface of the metal. A solution of 3-(1-chloroethyl) pyridine (2g) in anhydrous tetrahydrofuran (20cm^3) was allowed to drop slowly onto the stirred contents of the flask. After a time a yellow colouration was discernible which deepened to orange and eventually became red. When the addition was complete the mixture was refluxed for thirty minutes and then finely powdered isatin (1.1g, 0.5 mole equivalents) was added in a single portion. The mixture was refluxed for a further two hours and then lithium aluminium hydride (0.6g, 1.2 mole equivalents) was added. The mixture was refluxed for two more hours, and when cool the excess reducing agent was decomposed by the cautious addition of water. The product was isolated by extraction into ether (3x200cm^3), drying over anhydrous sodium sulphate and

filtering. The solvent was removed under reduced pressure to give an orange oil. This was taken up in a little chloroform and triturated with petroleum ether to give a buff-coloured solid which was collected at the pump. Analysis of this compound by TLC on silica in 5% methanolic chloroform solution showed several components, none of which corresponded with either starting materials or the desired 3-[1-(3-pyridyl) ethyl] indole. The isolated product did not have a sharp melting point and a satisfactory nuclear magnetic resonance spectrum could not be obtained.

I.R. νmax 3260 vb (O-H), 1715 (C=O) and 1600 (Ar)cm^{-1},

U.V. λmax 215,260,265 and 272 (sh) nm,

M.S. m/e (rel.int.%) 254 (M^+,3), 212(28), 210(39), 195(93) and 106(100)

Preparation of indole-3-yl-3-pyridyl methanone (111):

a. Preparation of potassium nicotinate:

Nicotinic acid (35g) was suspended in a mixture of ethanol ($100cm^3$) and water ($100cm^3$) and the mixture was stirred continuously while potassium hydroxide pellets (16g, 1 mole equivalent) were added in several portions. After completion of the addition the mixture was stirred for a further thirty minutes during which time the suspension cleared to a colourless solution. The solvents were removed under reduced pressure to give a white powder which was dried overnight in a vacuum oven at 110°C (44g, 96%).

b. Preparation of nicotinoyl chloride:

Finely ground dry potassium nicotinate (32g) in anhydrous toluene ($150cm^3$) was chilled in an ice bath while freshly distilled oxalyl chloride (25g)in anhydrous toluene ($50cm^3$) was added dropwise. The mixture was stirred for thirty minutes, brought to boiling over a further thirty minutes and maintained at reflux temperature for a

similar period. After cooling in an ice-salt bath the solution was used directly for reaction with Grignard reagents. This procedure is reported to effect an 80% conversion to the acid chloride[43].

c. Preparation of indol-3-yl-3-pyridyl methanone:

A solution of indolyl magnesium bromide (61g, 0.28 mole) in anhydrous ether (200cm^3) was added dropwise to a mechanically stirred solution of nicotinoyl chloride (0.19 mole) cooled in an ice-salt bath. The mixture was stirred overnight at room temperature when the organometallic complex was hydrolysed by the cautious addition of saturated ammonium chloride (30cm^3). The resulting dense yellow precipitate was filtered, washed with water and then ether, and recrystallised twice from ethanol to give the ketone as colourless leaflets. A second crop of product was obtained from the aqueous washings after basification and extraction with chloroform. (25.7g, 61%; 41% relative to indole)

^{13}C N.M.R. δ(d^6-DMSO)

2'	3'	3a	4'	5'	6'	7'	7a	CO
123.4d	124.5s	126.1s	121.5d	122.1d	115.2d	112.3d	136.9s	187.9s

2	3	4	5	6
151.7d	136.1s	135.8d	123.3d	148.9d

ppm,

^{1}H N.M.R. δ(d^6-DMSO) 8.94 (1H, d, J=2HZ, H-2),
8.77 (1H, dd, J=2HZ and J=5HZ, H-6),
8.36-8.08 (2H, complex, H-4 and H-4'),
8.02 (1H, s, H-2'),
7.65-7.20 (4H, complex, H-5, H-5', H-6' and H-7') ppm,

I.R. νmax 3150 (N-H), 1590 (C=O) and 1580 (Ar) cm^{-1},

UV λmax 257, 268 and 320nm,

M.S. m/e (rel.int.%) 222 (M^+, 81), 144 (100), 116(25) and 89(23),

m.p. 210-212° (lit.[43,44], 210-211°).

Preparation of 2-(3-pyridyl)-4,5-dihydroimidazole (107):

3-Cyanopyridine (31g, 0.3 mole) was stirred at 100° with 1,2-diaminoethane (20cm^3, 1 mole equivalent) in the presence of a catalytic amount of sulphur (1g) for four hours. The mixture was allowed to cool overnight to give a solid which was recrystallised from acetonitrile as white needles (39.7g, 90%).
m.p. 53° (lit.[59] 54°).

Preparation of 1,3-diacetyl-2-(3-indolyl)-2-(3-pyridyl) imidazoline (108):

The dihydroimidazole (107) (29.4g, 0.2 mole) was stirred for about thirty minutes in acetic anhydride (50cm^3) and then a solution of indole (23.4g, 1 mole equivalent) in acetic anhydride (50cm^3) was added with cooling. The solution was stirred overnight and the resulting solid product was filtered at the pump and washed with diethyl ether to give a light brown coloured solid (56.4g, 81%).
I.R. νmax 3150 (N-H) cm^{-1}.
m.p. 260°

Preparation of indol-3-yl-3-pyridyl methanone (111):

The imidazoline (108) (26.9g) was heated with sodium hydroxide (5g) in ethanol (125cm^3) and water (25cm^3) for two hours. The reaction mixture was poured onto ice (100g) and the title compound was filtered off as a colourless solid. Recrystallisation from ethanol gave the product as needles (14g, 80%).
m.p. 210-211° (lit.[43,44] 210-211°).

Preparation of indol-3-yl-3-pyridyl methanol (112):

Indol-3-yl-3-pyridyl methanone (111) (2.5g) was dissolved in ethanol (100cm^3) and the mixture was cooled in an ice bath. Sodium borohydride was added in small portions with stirring and the end point of the reaction was marked by the observation of a constant

ultra violet spectrum. Excess borohydride was decomposed by the dropwise addition of acetone. The solvent mixture was evaporated under reduced pressure and the residue was partitioned between water ($100cm^3$) and chloroform ($100cm^3$). The organic phase was separated, dried over magnesium sulphate, filtered and the solvent removed under reduced pressure to give a pale yellow oil which rapidly crystallised to a solid. This was recrystallised from ethanol to give colourless leaflets. (1.8g, 73%).

^{13}C N.M.R. $\delta(d^6$-DMSO)

2'	3'	3a	4'	5'	6'	7'	7a	CHOH
122.9d	118.8s	125.5s	119.2d	122.8d	118.4d	111.4d	140.9s	37.7d

2	3	4	5	6
148.0d	132.6s	133.8d	121.0d	147.6d

ppm,

^1H N.M.R. $\delta(d^6$-DMSO) 10.94 (1H,bs,NH),
8.69 (1H,d,J=2HZ,H-2),
8.45 (1H,dd,J=2HZ and J=5HZ, H-6),
7.85 (1H,dt,J=2HZ and J=8HZ,H-4),
7.60-6.84 (6H, complex, H-5,H-2',H-4',H-5',H-6' and H-7'),
6.07 (1H,d,J=4HZ,CHOH),
and 5.79 (1H,d,J=4HZ, CHOH) ppm,

I.R. νmax 3270 (O-H), 3140 (N-H), 1580 (Ar) and 995 (C-O) cm^{-1},

U.V. λmax 223,268,280 and 288 nm,

M.S. m/e(rel.int.%) 224 (M^+,89), 207(77), 206(100), 205(94), 146(32), 144(11) and 118(47),

m.p. 154°

Preparation of indol-3-yl-3-pyridyl methane (114):

Indol-3-yl-pyridyl methanone (111) (5.0g) in hot ethanol (>60°) was treated portionwise with sodium borohydride until a constant ultra violet spectrum was observed. The solution was

allowed to cool and excess reducing agent was decomposed by the addition of water ($10cm^3$). The solvent mixture was evaporated under reduced pressure and the residue was partitioned between water ($100cm^3$) and chloroform ($100cm^3$). The organic layer was separated, dried over magnesium sulphate, filtered and the solvent removed under reduced pressure to give an orange oil. Trituration with ether gave a solid which recrystallised from ethanol as near-colourless prisms (2.2g, 47%).

^{1}H N.M.R. δ(d^6-DMSO) 10.94 (1H,bs,NH),
8.56 (1H,d,J=2HZ,H-2),
8.32 (1H,dd,J=2HZ and J=5HZ, H-6),
7.60 (1H,dt,J=2HZ and J=5HZ, H-4),
7.48-6.84 (6H, complex, H-5,H-2',H-4',H-5',H-6' and H-7'),
and 4.17 (2H,s,CH_2)ppm,

I.R. νmax 3140 (N-H) and 1580 (Ar)cm^{-1},

U.V. λmax 235, 270, 284, and 292 nm,

M.S. m/e (rel.int.%) 208,(M^+,56) and 92(100),

m.p. 155-156° (lit.[43,44] 157-158°; lit.[51] 154-156°).

Preparation of 1-phenylsulphonylindol-3-yl-3-pyridyl methanone (127):

Under an atmosphere of dry nitrogen, crushed potassium hydroxide pellets (5.1g) were dissolved in dimethyl sulphoxide ($45cm^3$) which had been dried over molecular sieves. After stirring for ten minutes to form the anion, the presence of which was indicated by a slight pink colouration, indol-3-yl-3-pyridyl methanone (111) (5.0g) was added in a single portion. The mixture turned a deep red colour and was stirred for three hours to form the indole anion. The mixture was then placed in an ice bath and phenylsulphonyl chloride (8.0g , $6.0cm^3$) was added dropwise, maintaining the temperature below

20°. The solution was stirred for a further forty-five minutes and then distilled water ($30cm^3$) was added cautiously to prevent an increase in temperature. After fifteen minutes a further quantity of water ($75cm^3$) was added and a yellow precipitate formed which was filtered at the pump and washed copiously with diethyl ether. The residue was sucked dry and recrystallised from boiling methanol to give the product as yellow prisms (5.1g, 63%).

^{1}H N.M.R. δ(d^6-DMSO) 9.06 (1H,d,J=2HZ, H-2),
8.86 (1H,dd,J=2HZ and J=5HZ, H-6),
8.44 (1H, s,H-2'),
8.36-8.12 (4H, complex, H-4,H-7', H-2" and H-6"),
8.02 (1H,dd,J=1HZ and J=7HZ, H-5),
and 7.82-7.40 (6H, complex, H-4',H-5',H-6',H-3",H-4" and H-5") ppm,

I.R. νmax 1625 (C=O) and 1580 (Ar) cm^{-1},

U.V. λmax 208, 233 and 303 nm,

M.S. m/e (rel.int.%) 362 (M^+,67), 284(17), 221(46), 193(52), 144(28), 141(35) and 77(100),

m.p. 132-134°

(Found: C,66.6; H,4.0; N,7.8.

$C_{20}H_{14}N_2O_3S$ requires: C,66.3; H,3.9; N,7.7%).

Preparation of 1-acetylindol-3-yl-3-pyridyl methanone (128):

Indol-3-yl-3-pyridyl methanone (111) (3.0g) was treated with acetic anhydride ($15cm^3$) and the mixture refluxed for one hour. The excess reagent was removed under reduced pressure to leave a brown gum which was partitioned between saturated sodium hydrogen carbonate solution ($20cm^3$) and chloroform ($50cm^3$). The organic layer was separated, washed with water, dried over magnesium sulphate,

filtered and the solvent evaporated under reduced pressure to give an orange oil which slowly crystallised on standing. The product was recrystallised from ethanol to give the title compound as colourless prisms. (2.9g, 81%).

^{1}H N.M.R. $\delta(CDCl_3)$ 9.01(1H,d,J=2HZ,H-2),
8.77 (1H,dd,J=2HZ and J=5HZ, H-6),
8.42-8.02 (3H, complex, H-4,H-4' and H-7'),
7.83 (1H,s,H-2'),
7.54-7.30 (3H,complex H-5,H-5' and H-6'),
and 2.68 (3H,s,$COCH_3$)ppm,

I.R. νmax 1725 (N-$COCH_3$), 1615 (Ar_2C=O) and 1580 (Ar)cm^{-1},

U.V. λmax 208, 230, 252 and 312nm,

M.S. m/e (rel.int.%) 264 (M^+,70), 222 (79), 194(14) and 144(100).

m.p. 140° (lit.[104] 141°).

Preparation of 1-trifluoracetylindol-3-yl-3-pyridyl methanone (129):

Indol-3-yl-3-pyridyl methanone (111) (2.22g, 0.01 mole) and sodium trifluoracetate (1.36g, 0.01 mole) were dissolved in trifluoracetic anhydride (20cm^3) and the mixture was heated at reflux temperature for one hour. The excess solvent was removed by evaporation under reduced pressure and the residual gum was partitioned between saturated sodium hydrogen carbonate solution (20cm^3) and chloroform (40cm^3). The organic phase was separated, dried over magnesium sulphate, filtered and the solvent removed under reduced pressure to give an orange oil which slowly solidified. Recrystallisation from ethanol yielded a product which was identical in every respect to the starting ketone.

The same result was achieved if the reaction was repeated in the absence of sodium trifluoracetate.

Preparation of methyltriphenylphosphonium bromide:

Triphenylphosphine (10g, 0.038 mole) was dissolved in dry toluene ($10cm^3$) in a $100cm^3$ Erlenmeyer flask. The solution was cooled in an ice-salt bath and bromomethane ($4.9cm^3$, 0.05 mole) was quickly added. The flask was sealed with a bung secured by wire and was left to stand for a day at room temperature, during which time a white crystalline solid was deposited. Before opening, the flask was cooled in an ice-salt bath, then the solid was filtered off at the pump and washed with hot toluene. The product was dried in a vacuum oven at 50°C and stored in a desiccator over phosphorus pentoxide (12.5g, 92%).

Preparation of 1-(1-phenylsulphonylindol-3-yl)-1-(3-pyridyl) ethene (130):

Freshly-distilled tetrahydrofuran ($15cm^3$) was placed in a $100cm^3$ round-bottom, three-neck flask and was deoxygenated under a stream of dry nitrogen for about half an hour. Sodium hydride (4.8g, 50% suspension in oil) was then added and the mixture was stirred at 60° for one hour to aid dissolution of the sodium hydride. After this time the condenser was removed momentarily and methyltriphenyl-phosphonium bromide (2.8g, 1 mole equivalent) was added in portions over a period of fifteen minutes. The condenser was replaced and the mixture was stirred at room temperature for forty-five minutes to complete the formation of methylene triphenylphosphorane.

At the end of this period 1-phenylsulphonylindol-3-yl-3-pyridyl methanone (127) (2.5g, 1 mole equivalent) was added in a single portion. The pale yellow colour of the reaction mixture changed to deep brown. The mixture was then warmed and stirred for one hour at reflux before pouring into hydrochloric acid ($100cm^3$, 2M). The acid layer was washed with ether ($2x100cm^3$) , separated and basified with concentrated

ammonia solution ($25cm^3$). When cool, the aqueous layer was extracted with chloroform ($4x50cm^3$). The organic extracts were combined, dried over magnesium sulphate, filtered and the solvent removed under reduced pressure to give an orange oil.

Analysis of this compound by TLC on silica in ethyl acetate showed it to be a mixture of two major components which were separated by flash chromatography on a column of silica gel using a 50:50 mixture of ethyl acetate and 60-80° boiling fraction petroleum ether. The first fraction was dried over magnesium sulphate, filtered and the solvent removed under reduced pressure to give an orange oil which slowly solidified on standing. Repeated attempts to recrystallise this material from a variety of solvents were unsuccessful, but it was possible to identify it as the title compound by spectroscopic means (1.1g, 44%).

^{1}H N.M.R. δ(d^6-DMSO) 9.10 (1H,d,J=2HZ,H-2),
8.86 (1H,dd,J=2HZ and J=6HZ, H-6),
8.70-7.10 (12H, complex, remaining aromatic H's),
5.76 (2H,bs,CCH_2)ppm,

I.R. νmax 1610 (C=C)cm^{-1},

U.V. λmax 212, 262 and 296 nm,

M.S. m/e (rel.int.%) 360 (M^+)

Preparation of 1-acetyl-3-[1-(3-pyridyl)ethyl]indole (74):

3-[1-(3-Pyridyl)ethyl]indole (29) (3.4g) was heated under reflux in acetic anhydride ($20cm^3$) and triethylamine ($4cm^3$) for thirty minutes. The solvent was then removed under reduced pressure to give a dark oil which was dissolved in chloroform ($50cm^3$) and washed with saturated sodium hydrogen carbonate solution ($50cm^3$). The organic layer was separated, dried over magnesium sulphate, filtered and the solvent evaporated under reduced pressure to give

an orange oil which slowly crystallised on standing. The compound was recrystallised twice from ethanol to give an almost colourless solid. (2.9g, 71%).

^{1}H N.M.R. δ($CDCl_3$) 8.66(1H,d,J=2HZ,H-2),
8.60-8.36(2H, complex, H-6 and H-7'),
7.64-7.46(1H,dt,J=2HZ and J=8HZ,H-4),
7.42-7.08(5H,complex,H-5,H-2',H-4',H-5' and H-6'),
4.31(1H,q,J=7HZ,$CHCH_3$),
2.60(3H,s,$COCH_3$),
and 1.69(3H,d,J=7HZ,$CHCH_3$)ppm,

I.R. ν max 1700 (C=O)cm^{-1},

U.V. λmax 224, 257 and 316nm,

M.S. m/e (rel.int.%) 264(M^+,98), 222(85), 207(100) and 144(25),

m.p. 123° (lit.[43,44] 123-124°).

Preparation of 1-amino-3-[(1-acetylindol-3-yl)ethyl] pyridinium mesitylenesulphonate (75):

The acetylated indole (74) (2.6g) was dissolved in dichloromethane ($40cm^3$) and cooled to 0° in an ice bath. Mesitylenesulphonyl hydroxylamine (258)(2.1g, 1 mole equivalent) was also dissolved in dichloromethane ($40cm^3$) and cooled to 0°. The solutions were quickly mixed and stirred together for thirty minutes in an ice bath. After this time ice-cold anhydrous ether ($400cm^3$) was added and stirring was continued for a further hour during which time a pale yellow solid was precipitated. The solvent was decanted and evaporated under reduced pressure to give more of the yellow solid which was combined with the first crop.(4.5g,96%).

^{1}H N.M.R. δ($CDCl_3$) 9.10(1H,s,H-2),
8.71(1H,d,J=5HZ,H-6),
8.38(1H,d,J=9HZ,H-4),

7.80-7.04(6H, complex,H-5,H-2',H-4',H-5',H-6' and H-7'),
6.77(2H,s,ArH),
4.31(1H,q,J=7HZ,CHCH$_3$),
2.57(6H,s,ArCH$_3$),
2.51(3H,s,COCH$_3$),
2.18(3H,s,ArCH$_3$),
and 1.69(3H,d,J=7HZ,CHCH$_3$)ppm,

I.R. νmax 1700(C=O)cm^{-1},

U.V. λmax 207, 235, 260, 282, 292 and 301nm,

M.S. m/e (rel.int.%) The mass spectrum of this compound shows only peaks due to the starting compound, 1-acetyl-3-[1-(3-pyridyl)ethyl]indole (74).

Preparation of 1-acetylimido-3-[1-acetylindol-3-yl)ethyl]pyridine(76):

The pyridinium salt (75) (4.7g) was dissolved in chloroform (20cm^3), with warming if necessary, then cooled in an ice bath. Acetic anhydride (20cm^3) was also cooled to 0° and was quickly added and the mixture stirred together for fifteen minutes. The mixture was then neutralised by the addition of a solution of potassium carbonate (60g) in water (60cm^3). The phases were thoroughly mixed through vigorous stirring, then the mixture was filtered and extracted with chloroform (4x25cm^3). The organic extracts were combined, dried over magnesium sulphate, filtered and the solvent removed under reduced pressure without the aid of heat to give an unstable orange oil which was reacted immediately in the next step (4.0g,70%).

I.R. νmax 1700 (C=O) and 1560(N$^{\oplus}$-C=O)cm^{-1},

M.S. m/e (rel.int.%) 321 (M$^+$,28),264(46), 222(33) and 207(100).

Preparation of 3-[1-(1-acetylindol-3-yl)ethyl]-1-methylacetamido pyridinium iodide (77):

The aforementioned orange oil was dissolved in iodomethane (20 cm^3) and the mixture was refluxed for forty-five minutes. Excess

iodomethane was evaporated under reduced pressure to give a yellow foam which was triturated with acetone to give the product as a bright yellow solid (5.3g, 92%).

^{1}H N.M.R. δ(d^6-DMSO) 9.67(1H,s,H-2),
9.22(1H,d,J=5HZ,H-6),
8.73(1H,d,J=8HZ,H-4),
8.38(1H,dd,J=1HZ and J=9HZ,H-7'),
8.16(1H,dd,J=7HZ and J=8HZ,H-5),
7.75(1H,s,H-2'),
7.44-7.12(3H, complex, H-4',H-5' and H-6'),
4.73(1H,q,J=7HZ,CHCH$_3$),
3.81(3H,s,N-CH$_3$),
2.96(3H,s,N-COCH$_3$),
2.70(3H,s,$N^{\oplus}$-N-COCH$_3$),
and 1.87(3H,d,J=7HZ,CHCH$_3$)ppm,

I.R. νmax 1700(N-COCH$_3$) and 1695 ($N^{\oplus}$-N-COCH$_3$)cm^{-1},

U.V. λmax 203, 220, 239(sh), 265, 292 and 301nm,

(Found: C,51.9; H,4.65; N,8.9.

$C_{20}H_{22}N_3O_2I$ requires: C,51.8; H,4.75; N,9.1%).

Preparation of 4-cyano-3-[1-(1-acetylindol-3-yl)ethyl]-1,4-dihydro-1-methylacetamido pyridine:

The methiodide salt (77) (3.0, $6.5x10^{-3}$ mole) was dissolved in water ($150cm^3$) and ethanol ($10cm^3$) at 40°. Potassium cyanide (0.6g, $9.2x10^{-3}$ mole) and ammonium chloride (0.7g) were dissolved in water ($15cm^3$) and the solution was added dropwise to the stirred solution of the methiodide. The mixture was stirred for an hour at room temperature during which time a pale straw-coloured solid was precipitated. Chloroform ($15cm^3$) was added and stirring was continued for a further five minutes. The product was extracted into chloroform ($5x10cm^3$) and the extracts were bulked and washed copiously with

water. The organic layer was separated, dried over magnesium sulphate, filtered and the solvent removed under reduced pressure to give an orange oil. This procedure was carried out a number of times.

I.R. νmax 2220(C≡N) and 1700 (N-$COCH_3$)cm-1

Preparation of 1-acetyl-3-{1-[3-(4-cyano)pyridyl]ethyl} indole (78b):

The aforementioned oil was dissolved in ethanol ($50cm^3$) and stirred for two hours under a medium pressure ultra violet source. During this time an off-white solid was precipitated, which on subsequent investigation, proved to be regenerated 1-acetyl-3-[1-(3-pyridyl) ethyl]indole (74). The mixture was filtered and the filtrate evaporated under reduced pressure to give an orange oil which was chromatographed on a column of silica gel (Merck Kieselgel 60, 70-230 mesh) using chloroform as eluent. The first fractions from the column were combined and evaporated to dryness under reduced pressure to give an orange oil which slowly solidified on standing. The solid was collected at the pump and washed with ether. Recrystallisation from ethanol afforded the title compound as pale straw-coloured prisms (1.2g, 66%).

^{13}C N.M.R. δ(d^6-DMSO)

2'	3'	3a	4'	5'	6'	7'	7a	$\underline{C}HCH_3$
123.2d	115.6s	128.9s	123.3d	124.9d	118.6d	116.1d	141.5s	32.9d

$CH\underline{C}H_3$	2	3	4	5	6	$\underline{C}OCH_3$	$CO\underline{C}H_3$	$\underline{C}N$
20.5q	150.0d	135.4s	122.7s	124.6d	148.5d	186.9s	23.8q	169.2s

^1H N.M.R. δ($CDCl_3$) 8.62 (1H,d,J=2HZ,H-2),
8.60 (1H,d,J=6HZ,H-6),
8.39 (1H,d,J=8HZ,H-7'),
7.56-7.10 (5H, complex, H-5,H-2', H-4', H-5' and H-6'),

4.69 (1H,q,J=8HZ,$C\underline{H}CH_3$),
2.70 (3H,s,$COCH_3$),
and 1.84 (3H,d,J=8HZ,$CHCH_3$)ppm,

I.R. ν max 2220 (C≡N) and 1700 ($N\text{-}COCH_3$)cm^{-1},

U.V. λ max 207, 226, 239, 265, 292 and 300 nm,

M.S. m/e (rel.int.%) 289 (M^+, 34), 247(78) and 232(100),

m.p. 110° (lit.[42] 111-112°).

Preparation of 5-allyl-5-demethylellipticine (169):

a. Reaction between 1-acetyl-3-{1-[3-(4-cyano)-pyridyl]ethyl} indole (78b) and allylmagnesium bromide:

Allylmagnesium bromide was prepared by the action of a solution of allyl bromide (0.23g) in anhydrous diethyl ether ($30cm^3$) upon a vigorously stirred suspension of magnesium turnings (0.37g) in dry ether ($20cm^3$) at -15°.

The cyanide compound (78b) (50mg) was dissolved in freshly distilled anhydrous tetrahydrofuran ($20cm^3$) and this solution was allowed to drop slowly onto the chilled Grignard reagent with stirring. On completion of the addition the mixture was allowed to warm to room temperature and was stirred for a further hour. After this time the contents of the flask were poured onto crushed ice and the mixture was extracted with chloroform ($2x50cm^3$). The organic extracts were combined, dried over magnesium sulphate, filtered and the solvent removed under reduced pressure to leave a brown gum which showed two major spots on TLC. One of these corresponded to the unchanged nitrile (78b).

M.S. m/e (rel.int.%) 314(M^+,17), 289(M^+,35), 247(58) and 232(100).

b. When this reaction procedure was repeated on a larger scale, a single component was isolated from the reaction mixture which was identified as the starting nitrile.

c. Preparation of allyllithium:

Freshly distilled anhydrous tetrahydrofuran ($50cm^3$) and freshly-cut lithium pieces (4.2g) were cooled in an ice-salt bath while a solution of allylphenyl ether (6.7g) in dry ether ($25cm^3$) was added dropwise with rapid stirring. To aid initiation of the reaction a little biphenyl was added. After about forty-five minutes the addition was complete and the cooling bath was removed whilst stirring continued for a further fifteen minutes. The resulting deep red solution was then decanted through glass wool from the remaining lithium metal and portions were analysed by the usual titration technique[94]. The concentration of the allyllithium solution was found to be 0.3M which corresponded to a yield of 45% relative to allylphenyl ether.

d. Reaction between 1-acetyl-3-{1-[3-(4-cyano)pyridyl]ethyl} indole (78b) and allyllithium:

The cyanide compound (78b) (500mg) was dissolved in freshly distilled anhydrous tetrahydrofuran ($50cm^3$) and the solution was added dropwise to a stirred solution of allyllithium ($20cm^3$, 0.3M, 3.5 mole equivalents) in tetrahydrofuran cooled in an ice-salt bath. Addition was completed after about thirty minutes and the resulting deep brown mixture was stirred overnight under nitrogen. Excess organometallic reagent was decomposed by the addition of a solution of ammonium chloride (10g) in water ($50cm^3$). The product was extracted into chloroform, dried over magnesium sulphate, filtered and the solvent removed under reduced pressure to give a brown oil.

I.R. νmax 1660 (C=N) cm^{-1},

M.S. m/e (rel.int.%) 289(M^+,100).

e. Preparation of 5-allyl-5-demethylellipticine (169):

The crude product from the previous reaction was dissolved in acetic acid ($40cm^3$, 30%) and refluxed overnight. When cool, the reaction mixture was neutralised with solid potassium carbonate and extracted with chloroform ($3x50cm^3$). The combined organic extracts were dried over magnesium sulphate, filtered and the solvent removed under reduced pressure to give a viscous brown oil. This was taken up in a little chloroform and triturated with diethyl ether to give an amorphous dark green-coloured solid which was collected at the pump. Attempts to recrystallise this material from a range of solvents were unsuccessful. On TLC the material showed a number of spots, one of which fluoresced strongly under ultra violet light and which did not move far up the plate in solvents of medium polarity. The crude product was then taken up in chloroform and chromatographed on a column of silica gel. This improved its purity by only a small amount, the result being an orange-coloured solid which could still not be induced to crystallise.

U.V. λmax 209(sh), 224, 278, 292 and 298 nm,

M.S. m/e (rel.int.%) 272 (M^+,70), 257(49), 245(79) and 232(100).

f. When the reaction between 1-acetyl-3-{1-[3-(4-cyano)pyridyl] ethyl}indole (78b) and allylithium was repeated at -78° the same oily intermediate imine was obtained, which was hydrolysed in the manner described above to give a brown solid. This resembled the product obtained in the first reaction in all its spectral features, and defied attempts to purify it either by recrystallisation or by chromatographic means.

Attempted preparation of 5-demethyl-5-(3,3-diethylamino)propylamino-ellipticine (171):

a. Lithiation of 3,3-diethylaminopropylamine:

Freshly cut lithium pieces (0.2g) were stirred into dry benzene ($20cm^3$) and then freshly-distilled hexamethylphosphoric triamide (HMPT) ($20cm^3$) was added. A solution of 3,3-diethylaminopropylamine (3.0g) in dry benzene ($10cm^3$) was then added dropwise to the stirred mixture in the flask at room temperature. No reaction was apparent, so the reaction vessel was warmed and the contents gently brought to boiling whilst stirring was maintained for a couple of hours. After this time a distinct pink colouration was discernible, but it was felt that a more satisfactory preparation of the lithium derivative could be achieved using n-butyllithium.

b. Lithiation of 3,3-diethylaminopropylamine:

Dry benzene ($20cm^3$) and freshly-distilled HMPT ($20cm^3$) were added to 3,3-diethylaminopropylamine (4.7g) in a nitrogen-purged flask. The mixture was cooled in a bath of acetone and solid carbon dioxide. Butyllithium in hexane solution ($30cm^3$, 1.3M) was added in two equal portions to the stirred mixture in the flask, giving rise to an orange colouration. Stirring was continued for a couple of hours after which time the cooling bath was removed and the contents of the flask were stirred at room temperature for half an hour. Portions of the organometallic reagent were removed for titration which revealed that its concentration was 0.31M, corresponding to a 60% conversion to the lithiated amine.

c. Reaction between 1-acetyl-3-{1-[3-(4-cyano)pyridyl]ethyl}indole (78b) and the lithium salt of 3,3-diethylaminopropylamine:

The organometallic reagent from the above reaction ($20cm^3$, 0.31M, 3.5 mole equivalents) was cooled in an ice-salt bath and a solution

of the nitrile (78b) (500mg) in anhydrous tetrahydrofuran ($50cm^3$) was added dropwise to the stirred contents of the flask over a period of thirty minutes. The mixture turned a deep red colour and was allowed to warm up slowly whilst stirring overnight under nitrogen.

A solution of ammonium chloride (10g) in distilled water ($50cm^3$) was added cautiously to decompose excess reagent and the mixture was extracted with chloroform ($3x50cm^3$), dried over magnesium sulphate, filtered and the solvent evaporated under reduced pressure to leave a mobile orange oil which was probably the desired imine dissolved in HMPT.

I.R. νmax 3480, 2860 (C-H), 1460, 980 and 740 cm^{-1}, (all HMPT),

U.V. λmax

M.S. m/e (rel.int.%) 377 (M^+,82), 289(M^+,29), 247(94) and 232(100).

d. Attempted preparation of 5-demethyl-5-(3,3-diethylamino) propylaminoellipticine (171):

The oil from the above reaction was dissolved in acetic acid ($50cm^3$, 50%) and heated at gentle reflux overnight. When cool, the mixture was made basic with solid potassium carbonate and extracted into chloroform ($2x50cm^3$). The combined extracts were dried over magnesium sulphate, filtered, and the chloroform removed under reduced pressure to leave an orange oil. This was then evaporated further under high vacuum to leave a viscous brown oil. Analysis of this material by TLC showed it to be mainly a single component. Purification was achieved by eluting the material through a column of silica gel using chloroform as the eluent, to give an amorphous brown solid. Spectral analysis then revealed that the compound was the de-acetylated nitrile (78a).

I.R. νmax 3270 (N-H), 2220 (C≡N) and 1580 (Ar) cm^{-1},

M.S. m/e (rel.int.%) 247(M^+,87), 246(100) and 232(17).

Preparation of 5-demethyl-5-(4-picolyl)ellipticine (173):

a. Preparation of 4-picolyllithium:

Freshly distilled anhydrous tetrahydrofuran ($20cm^3$) and 4-picoline (2.0g, 0.0215 mole) were stirred together and cooled in an ice-salt bath. A solution of n-butyllithium in hexane ($15cm^3$, 0.0215 mole) was added dropwise over a period of thirty minutes with stirring. The cooling bath was removed and the solution allowed to stir for a further hour while warming to room temperature. The resulting solution of 4-picolyllithium was a deep yellow colour with some precipitated solid. A portion of this material was extracted and titrated in the usual manner[94] to determine its concentration.

b. Reaction between 1-acetyl-3-{1-[3-(4-cyano)pyridyl]ethyl}indole (78b) and 4-picolyllithium:

The nitrile (78b) (500mg, $1.73x10^{-3}$ mole) was dissolved in freshly distilled dry tetrahydrofuran ($50cm^3$). The solution was added to the slurry of 4-picolyllithium with vigorous stirring over a period of about fifteen minutes at room temperature, using a bath of cold water to prevent elevation of temperature. The resulting brown solution was stirred for a further hour, then the excess organo-metallic reagent was decomposed by the cautious addition of iced water ($10cm^3$), followed by a solution of ammonium chloride (10g) in water ($50cm^3$). The organic phase was separated and the aqueous layer extracted with chloroform ($2x50cm^3$). The extracts were combined and dried over magnesium sulphate, filtered and the solvent removed under reduced pressure to give an orange oil which smelled strongly of 4-picoline.

I.R. νmax 3200 (N-H) and 1600 (Ar)cm^{-1}

M.S. m/e (rel.int.%) 340 (M^+, 67) and 289(100).

c. Preparation of 5-demethyl-5-(4-picolyl) ellipticine (173):

The aforementioned oil was dissolved in acetic acid ($20cm^3$, 20%) and heated on a steam bath for one hour. After this time it was allowed to cool before basification with solid potassium carbonate. The mixture was extracted with chloroform ($4x50cm^3$) and the extracts bulked and dried over magnesium sulphate. The solution was filtered and the solvent evaporated under reduced pressure to leave an orange oil. Analysis of this material by TLC indicated that it comprised several components, one of which fluoresced strongly under ultra violet light. Trituration of the oil with diethyl ether afforded a light brown solid which was resistant to attempts at recrystallisation from a variety of solvents. Column chromatography on silica gel using chloroform as eluent failed to yield a pure component. Finally, the crude product was applied to a preparative silica plate and chromatographed in a solvent mixture of ethyl acetate and 60-80° boiling fraction petroleum ether (50:50 mixture). The fluorescent band was removed and extracted from a Soxhlet thimble using ethyl acetate. Evaporation of the solvent under reduced pressure gave a small quantity of fine yellow needles (4mg,<1%).

^{1}H N.M.R. δ(d^6-DMSO) 11.68 (s,NH),
Fourier Transform; 9.76 (s,H-1),
no integral 8.60-7.08 (aromatic H's),
4.73 (s,Ar-CH_2-Ar),
and 2.48 (s,CH_3)ppm,

UV λmax 208, 280, 292 and 297 nm,
M.S. m/e (rel.int.%) 323 (M^+,100) and 245 (51).

d. Preparation of 5-n-butyl-5-demethylellipticine (174):

When the above procedure was repeated using a freshly prepared suspension of 4-picolyllithium, extraction of the hydrolysis product with chloroform gave a bright yellow solution. Evaporation of the solvent to low bulk gave a solid product as yellow needles which were collected at the pump. Spectroscopic analysis of this material showed it to be the title compound.

Preparation of 5-n-butyl-5-demethylellipticine (174):

a. Reaction between 1-acetyl-3-{1-[3-(4-cyano)pyridyl]ethyl}indole (78b) and n-butyllithium:

The cyanide compound (78b) (500mg) was dissolved in freshly distilled anhydrous tetrahydrofuran ($100cm^3$). This was added dropwise to a stirred solution of n-butyllithium in hexane ($3.5cm^3$, 1.6M, 3 mole equivalents) cooled in an ice-salt bath. After the completion of the addition, stirring was continued for a further forty-five minutes, then iced water ($10cm^3$) was added, followed by an aqueous solution of ammonium chloride ($50cm^3$, 20%). The organic phase was separated, dried over sodium sulphate, filtered and the solvent removed under reduced pressure to give an orange gum.

b. Preparation of 5-n-butyl-5-demethylellipticine (174):

The aforementioned gum was heated with acetic acid ($20cm^3$, 20%) on a steam bath for an hour. The solution was then cooled, made basic with solid potassium carbonate and extracted with chloroform ($5x10cm^3$). The extracts were bulked, dried over magnesium sulphate, filtered and most of the solvent removed under reduced pressure, giving the title compound as yellow needles which were recrystallised from methanol (320mg, 64%).

^{1}H N.M.R. δ(TFA-d) 11.25 (1H,s,NH),
9.55 (1H,s,H-1),
8.44-8.12 (4H, complex, H-3, H-4, H-7 and H-10),
7.62 (2H, complex, H-8 and H-9),
3.28 (3H,s,ArCH$_3$),
3.20 (2H,t,J=8HZ,Ar-CH$_2$-CH$_2$-),
1.92-1.62 (4H,m,-CH$_2$-CH$_2$-),
and 1.05 (3H,t,J=7HZ,-CH$_2$-CH$_3$) ppm,

I.R. ν max 3150 (N-H), 1605(Ar) and 1600 (Ar)cm^{-1},

U.V. λ max 240, 277, 288 and 296nm,

M.S. m/e (rel.int.%) 288 (M^+,56) and 245(100).

m.p. 280-284° dec. (lit.[44] 285-287° sublimes).

Preparation of 1-amino-3-(1-phenylsulphonylindol-3-ylformyl) pyridinium mesitylenesulphonate (187):

The indole (127) (10g) was dissolved in dichloromethane (100cm^3) and cooled to 0°. The aminating agent (258) (6.6g, 1 mole equivalent) was also dissolved in dichloromethane (50cm^3) and the two solutions were quickly mixed and stirred at the depressed temperature for half an hour. Ice-cold sodium-dried ether (500cm^3) was then added and stirring continued for a further hour during which time a pale yellow solid was precipitated. The mixture was filtered and the solvent evaporated under reduced pressure to give more of the yellow compound which was combined with the first crop. (16.2g, 97%).

^{1}H N.M.R. δ(d^6-DMSO) 9.17 (1H,d,J=2HZ,H-2),
9.01 (1H,dd,J=2HZ and J=5HZ,H-6),
8.75 (1H,s,H-2'),
8.72-8.50 (2H, complex,
8.36-7.90 (4H, complex,
7.80-7.14 (5H, complex,

6.76 (2H,s,aryl protons of mesitylenesulphonate anion),
3.39 (2H,bs,$\underline{NH_2}$),
2.54 (6H,s,$\underline{CH_3}$),
and 2.08 (3H,s,$\underline{CH_3}$)ppm,

I.R. νmax 3280 and 3220 (N-H) and 1640 ($Ar_2C{=}O$)cm^{-1}

U.V. λmax 207, 225, 262 (sh) and 315nm,

M.S. m/e (rel.int.%) 362(M^+,67), 284(19), 221(49), 193(51) and 77(100).

m.p. 254-256°.

Reaction of the salt (187) with acetic anhydride:

The mesitylenesulphonate (187) from the previous reaction was partitioned between water (100cm^3) and chloroform (150cm^3), and the mixture was cooled in an ice bath. Ice-cold acetic anhydride (50cm^3) was quickly added and the contents of the flask were stirred together for about fifteen minutes. Neutralisation was then carried out by the addition of a solution of potassium carbonate (120g) in distilled water (120cm^3). The phases were thoroughly mixed through vigorous stirring, then the mixture was filtered and extracted with chloroform (4x50cm^3). The organic extracts were combined, dried over magnesium sulphate, filtered and the solvent removed under reduced pressure in a cold water bath to give an orange oil which was used straight away in the next stage.

Preparation of 1-(N-acetyl-N-methylamino)-3-(1-phenylsulphonylindol-3-ylformyl)pyridinium iodide (189):

The aforementioned orange oil was added to iodomethane and the mixture was gently refluxed for an hour. Excess iodomethane was removed under reduced pressure to give an orange oil which was triturated with acetone to give the title compound as a bright yellow solid. (5.8g, 65% relative to (187)).

^{1}H N.M.R. δ(d^6-DMSO) 9.89 (1H,s,H-2),
9.56 (1H,d,J=6HZ,H-6),
9.22 (1H,d,J=7HZ,H-4),
8.76-7.30 (11H, complex, remaining aromatic H's),
3.86 (3H,s,NCH_3),
and 1.95 (3H,s,$NCOCH_3$)ppm,

I.R. νmax 1695 ($NCOCH_3$) and 1645 ($Ar_2C=O$)cm^{-1}

U.V. λmax 219, 262 and 300nm,

M.S. sample not volatile

m.p. 234-237° dec.

(Found: C,49.2; H,3.5; N,7.4.

$C_{23}H_{20}N_3O_4IS$ requires: C,49.2; H,3.6; N,7.5%).

Attempted preparation of 1-phenylsulphonylindol-3-yl-3-[1-N-acetyl-N-methylamino)-4-cyano-1,4-dihydro]pyridyl methanone (190):

The methiodide compound (189) (3.0g) was suspended in a mixture of warm water (150cm^3) and ethanol (10cm^3) at 40°. A solution of potassium cyanide (0.6g) and ammonium chloride (0.7g) in water (15cm^3) was then added dropwise with stirring. The mixture was stirred for one hour, then chloroform (10cm^3) was added and stirring was continued for a further ten minutes. The organic layer was separated and the aqueous phase was extracted with additional portions of chloroform (5x25cm^3). The combined organic extracts were dried over magnesium sulphate, filtered and the solvent removed under reduced pressure to give an orange oil. (1.9g, 77%).

^{1}H N.M.R. δ($CDCl_3$) 8.26 (1H,m,H-7'),
8.10-7.08 (4H, complex, H-2", H-6", H-6' and H-5'),
7.67-7.32 (5H, complex, H-2, H-3", H-4", H-5" and H-4'),
7.28 (1H,s,H-2'),
6.74 (1H,m,H-6),

5.21 (1H,m,H-5),
4.76 (1H,m,H-4),
3.31 (3H,s,NCH_3)
and 1.94 (3H,s,$NCOCH_3$)ppm,

I.R. νmax 2230 and 2220 (C≡N), 1680($NCOCH_3$) and 1625 ($Ar_2C=O$)cm^{-1},

U.V. λmax 211, 240, 300 and 315nm,

M.S. m/e (rel.int.%). 387(M^+,64), 362(100); only ions due to the fully aromatic nitrile observed.

Attempted aromatisation of the dihydropyridine (190):

The oil from the previous reaction was dissolved in ethanol ($50cm^3$) and stirred overnight under a medium pressure ultra violet source. The solvent was then removed under reduced pressure to give an orange oil which was chromatographed on a column of silica gel using chloroform as eluent. The first fractions were combined, dried over magnesium sulphate and the solvent evaporated at reduced pressure to give another orange oil. This was surprising, since the anticipated product was expected to be a solid. Analysis of the material by TLC showed it to be significantly more pure than the crude reaction product, giving rise to a spot which was also present in the original oil.

^1H N.M.R. δ(d^6-DMSO) 8.56 (1H,m
8.50-7.80 (4H, complex,
7.76-7.28 (5H, complex,
6.76 (1H,dd,J=16HZ and J=7HZ,H-4),
6.12 (1H,s,H-1),
3.27 (3H,s,NCH_3),
and 2.19 (3H,s,$NCOCH_3$)ppm,

I.R. ν max 2220 (C≡N),1680 ($NCOCH_3$) and 1625 ($Ar_2C=O$)cm^{-1},

U.V. λ max 210, 242, 268 and 300nm,

M.S. m/e (rel.int.%) 387(M^+, 38) and 362 (100).

Since it was apparent that aromatisation had not taken place as expected, a second portion of the dihydropyridine (190) was dissolved in ethanol and refluxed gently for a couple of hours. Preliminary investigation of the reaction mixture by TLC indicated that no change had taken place and when the solvent was removed under reduced pressure, unmodified starting material was returned.

Attempted preparation of 10H-indolo [3,2-b]-2-azaindene (207):

a. Attempted preparation of indolo[3,2-b] pyrido[4,3-d] cyclopentanone (215a):

Indol-3-yl-3-pyridyl methanone (111) (2.22g, 0.01 mole) and palladium acetate (0.11g, 0.005 mole) were dissolved in glacial acetic acid (40cm^3) under an atmosphere of dry nitrogen. The mixture was gently refluxed for forty hours and was then filtered through Kieselguhr. The solvent was evaporated under reduced pressure to give a dark orange gum which was chromatographed on a column of silica gel using chloroform as the eluent. The combined fractions were dried over magnesium sulphate, filtered and the solvent removed under reduced pressure to give a buff-coloured solid. This was found to be identical with the starting ketone (111).

b. Reaction between indol-3-yl-3-pyridyl methanone and hydrogen peroxide:

The indole (111) (2.22g, 0.01 mole) was dissolved in glacial acetic acid (20cm^3) and hydrogen peroxide solution (2.0cm^3, 35%) was added. The mixture was immersed in a water bath at a constant 70°. After three hours a further portion of the hydrogen peroxide solution (1cm^3, 35%) was added and the mixture was maintained at the elevated temperature overnight. The solution was then concentrated to about 10cm^3 by evaporation under reduced pressure, diluted

with water ($10cm^3$) and then evaporated as far as possible. The residue was made strongly alkaline by the addition of aqueous sodium carbonate and was then shaken with chloroform and left to stand. The mixture was filtered and the organic layer separated and dried over magnesium sulphate. Filtration and evaporation of the solvent under reduced pressure gave an amorphous brown solid. Analysis by TLC showed this material to be a multi-component mixture. Flash chromatography on a column of silica gel afforded unreacted starting material and an unidentified yellow solid.
M.S. m/e (rel.int.%) 238(M^+,6), 222(M^+,100), 194(21) and 144(98).

Preparation of ellipticine (1a):

1-Acetyl-3-{1-[3-(4-cyano)pyridyl]ethyl}indole (78b) (500mg, $1.73x10^{-3}$ mole) was dissolved in freshly distilled dry tetrahydrofuran ($100cm^3$). This was added dropwise to a solution of methyllithium in hexane ($3.8cm^3$,1.38M, 3 mole-equivalents) at -10° under an atmosphere of dry nitrogen with stirring. After completion of the addition stirring was continued for thirty minutes, then iced water ($10cm^3$) was cautiously added, followed by a solution of ammonium chloride (10g) in water ($50cm^3$). The organic phase was separated, dried over sodium sulphate, filtered and the solvent removed under reduced pressure to give a gum. This was heated on a steam bath with acetic acid ($20cm^3$, 20%) for one hour before allowing to cool. The solution was rendered basic with solid potassium carbonate and the product was extracted into chloroform ($4x50cm^3$). The extracts were combined, dried over magnesium sulphate, filtered and then evaporated to low bulk to furnish ellipticine as bright yellow needles. (350mg,82%).

^{13}C N.M.R. δ (d^6-DMSO)

1	3	4	4a	5	5a	6a	7	8
149.7d	140.5d	123.7d	132.5s	107.9s	142.7s	140.4s	110.7d	115.8d
9	10	10a	10b	11	11a	12	13	
127.1d	123.4d	121.9s	123.1s	127.8s	126.9s	14.3q	11.9q	ppm

^1H N.M.R. δ (d^6-DMSO) 11.22 (1H,s,NH),
9.56 (1H,s,H-1),
8.44-8.20 (2H, complex, H-3 and H-10),
7.79 (1H,d,J=6HZ, H-4),
7.56-7.44 (2H, complex, H-7 and H-9),
7.30-7.18 (1H, complex, H-8)
3.22 (3H,s,$C_{(11)}$-CH_3),
and 2.77 (3H,s,$C_{(5)}$-CH_3) ppm

I.R. νmax 3150 (N-H) and 1590 (Ar)cm^{-1}

U.V. λmax 239, 277, 287 and 295nm,

M.S. m/e (rel.int.%) 246 (M^+,100) and 231(25).

m.p. 309-312° (lit.[1] 311-315°).

Reaction between 1-methylindol-3-ol acetate (236) and 4-acetyl-3-ethylpyridine (237):

The indoxyl ester (236)(2.8g) was placed in a 250cm^3 Erlenmeyer flask previously purged with oxygen-free nitrogen. A solution of 4-acetyl-3-ethylpyridine (237)(2.2g) was prepared in aqueous methanol (35cm^3, 50%) containing potassium hydroxide (7.5g) and this was degassed for about half an hour. The solution was then added to the indoxyl ester and the flask, tightly stoppered, was left to stand for one week under nitrogen.

After this time the solution was filtered under nitrogen and the solvents were removed under reduced pressure to leave a dark gum. This was extracted with chloroform (3x100cm^3) and the combined

extracts were dried over magnesium sulphate, filtered and the solvent evaporated under reduced pressure to give a deep blue-coloured amorphous solid. Analysis of this material by TLC indicated that it contained two major components: the blue compound remained almost stationary on the plate whilst the second orange component was much more mobile.

The material was chromatographed on a column of silica gel using chloroform, and whilst this procedure was successful in separating the orange substance from the blue material, it could not be induced to crystallise. Further column chromatography was carried out in an attempt to resolve the orange material into its components, but with little success.

I.R. ν max 1700 (C=O) and 1645 (C=C)cm^{-1}

The amorphous orange solid (1g) was dissolved in ethanol ($50cm^3$) and treated portionwise with sodium borohydride until a constant ultra violet trace was obtained. The solvent was removed under reduced pressure and the residue was partitioned between chloroform ($50cm^3$) and water ($50cm^3$). The organic layer was separated, dried over magnesium sulphate, filtered and the solvent removed under reduced pressure to give an orange oil which slowly solidified on standing. Attempts to recrystallise this material from a variety of solvents were unsuccessful. A portion of the compound was flash-chromatographed on a column of silica gel using a solvent mixture of ethyl acetate and 60-80° boiling fraction petroleum ether (50:50), but subsequent treatment of the column extracts failed to yield a single component which could be recrystallised.

Preparation of N-methylanthranilic acid (246):

a. From anthranilic acid and iodomethane:

Anthranilic acid (13.7g, 0.1 mole) was suspended in water ($50cm^3$) and anhydrous potassium carbonate was added with stirring until a clear orange solution was obtained. Iodomethane (14.2g, 0.1 mole) was added and the mixture was refluxed overnight, during which time the product was precipitated as a mass of near-colourless needles. The reaction mixture was filtered and the residue recrystallised from aqueous alcohol to give the title compound as colourless needles (9.7g, 64%).

^1H N.M.R. $\delta(CDCl_3)$ 7.96 (1H,dd,J=2HZ and J=9HZ, H-2),
7.27 (1H,m,H-5),
6.76-6.46 (2H, complex, H-3 and H-4),
and 3.55 (3H,s,NCH_3)ppm,

I.R. νmax 3380 (N-H), 3300-2400 (O-H, associated), 1660 (C=O) and 1580(Ar)cm^{-1}

m.p. 178-179° (lit.[120] 182°).

b. From anthranilic acid and dimethyl sulphate:

Anthranilic acid (13.7g, 0.1 mole) was suspended in water ($40cm^3$) and a solution of sodium hydroxide (10g) in water ($30cm^3$) was added and the mixture brought to the boil with stirring. The heat source was removed as dimethyl sulphate ($12cm^3$, 0.125mole) was added at such a rate that the mixture was maintained at gentle reflux. Heating was then continued for half an hour and then sodium hydroxide (1.5g) in water ($5cm^3$) was added to make the reaction mixture slightly alkaline. A further portion of dimethyl sulphate ($6.0cm^3$) was added and this procedure was repeated until a total volume of $24cm^3$ of dimethyl sulphate had been used. Finally, the reaction mixture was made strongly alkaline by the addition of sodium hydroxide pellets, and was left to cool. The product was precipitated

as pale yellow needles which were filtered at the pump and recrystallised from aqueous alcohol. (9.5g, 63%).

Preparation of N-methylphenylglycine-o-carboxylic acid (247):

a. N-Methylanthranilic acid (246) (5.0g) and chloroacetic acid (3.7g) were stirred together in saturated sodium hydrogen carbonate solution ($40cm^3$). A catalytic amount of copper bronze (0.1g) was added and the mixture was refluxed for twenty-four hours. When cool, the mixture was filtered to give a green solution. The pH was adjusted to about 3 using hydrochloric acid (36%). The solution was then evaporated to low bulk and when cool the precipitated product was filtered, washed with water and dried in air. Recrystallisation from aqueous methanol afforded the title compound as a light tan solid. (3.2g, 46%).

b. N-Methylanthranilic acid (246) (5.0g) was suspended in water ($5cm^3$) and sodium hydroxide (1.5g) was added. In a separate vessel, chloroacetic acid (3.7g) was dissolved in water ($5cm^3$) and neutralised with anhydrous sodium carbonate (2.0g). The two solutions were warmed to 40° in a thermostatic water bath, and then quickly mixed and left to stand for twenty-four hours at the elevated temperature. When cool, the product was present as a semi solid mass which was dissolved in water ($50cm^3$) containing sodium hydroxide (2.0g). Acidification with concentrated hydrochloric acid gave the desired compound as a buff-coloured precipitate which was collected at the pump, washed with ether and allowed to dry in air. (5.7g, 75%).
I.R. ν max 3300-2400 (O-H,associated),1725($CH_2CO.OH$),1680(ArCO.OH) and 1600 (Ar)cm^{-1}

Preparation of 1-methylindol-3-ol-acetate (236):

a. N-Methylphenylglycine-o-carboxylic acid (247)(5.0g) was heated under reflux with sodium acetate (3.8g) in acetic anhydride ($75cm^3$) for three hours. After this time excess solvent was evaporated under reduced pressure to leave a brown solid which was dissolved in methanol and decolourised with charcoal. The alcoholic solution was then exhaustively extracted with hot petroleum ether (60-80° boiling fraction). The petrol extracts were combined and reduced in volume, then left to cool. The indoxyl ester (236) crystallised as yellow prisms (2.8g, 62%).

b. When the above procedure was repeated using triethylamine ($10cm^3$) instead of sodium acetate, the yield of 1-methylindol-3-ol acetate was increased to 3.2g (71%).

^{1}H N.M.R. δ($CDCl_3$) 7.49 (1H,dd,J=2HZ and J=8HZ, H-7),
7.23-6.81 (4H, complex, H-2,H-4,H-5 and H-6),
3.54 (3H,s,NCH_3),
and 2.22 (3H,s,$COCH_3$)ppm,

I.R. νmax 1735 (C=O) and 1220 (C-O) cm^{-1},

U.V. λmax 223 and 288nm,

M.S. m/e (rel.int.%) 189 (M^+,37), 132(100) and 115(23),

m.p. 58-59° (lit.[120] 59°)

Preparation of 4-cyano-3-ethylpyridine (248):

a. Preparation of 1-amino-3-ethylpyridinium mesitylenesulphonate (250):

3-Ethylpyridine (20g) was dissolved in dichloromethane ($45cm^3$) and cooled in an ice bath. Mesitylenesulphonyl hydroxylamine (258) (40.2g , 1 mole equivalent) was also dissolved in dichloromethane ($90cm^3$) and chilled. The two solutions were then quickly mixed and stirred at the depressed temperature for thirty minutes.

The mixture was then poured into ice-cold anhydrous diethyl ether ($800cm^3$) and stirred for ninety minutes, during which time a pale yellow oil separated. The ether was decanted and evaporated under reduced pressure to yield more of the yellow oil which was combined with the first batch. (56g, 93%).

b. Preparation of 3-ethylpyridine-N-acetylimide (251):

1-Amino-3-ethylpyridinium mesitylenesulphonate (250) (10g) was dissolved in water ($15cm^3$) and cooled to below 5°. Acetic anhydride ($30cm^3$) was then added and the mixture stirred vigorously for ten minutes. A solution of potassium carbonate (80g) in water ($80cm^3$) was then added and the reactants thoroughly mixed. The contents of the flask were then filtered and the solution was extracted with chloroform ($3x50cm^3$). The extracts were combined, dried over magnesium sulphate, filtered and the solvent removed under reduced pressure in a cold water bath to give a yellow oil which was immediately used in the next step.

c. Preparation of 1-(N-acetyl-N-methylamino)-3-ethylpyridinium iodide (252):

The aforementioned yellow oil was added to iodomethane ($15cm^3$) and the mixture was refluxed for forty-five minutes. After this time excess iodomethane was evaporated under reduced pressure to leave an orange oil which could not be induced to crystallise, either by refrigeration or by scratching. (6.0g,63%).

I.R. νmax 1700 ($NCOCH_3$) cm^{-1}.

d. Preparation of 1-(N-acetyl-N-methylamino)-4-cyano-1,4-dihydro-3-ethylpyridine:

The methiodide compound (1.9g) was dissolved in water ($30cm^3$) and treated with a mixture of ammonium chloride (0.7g) and potassium

cyanide (0.6g, 1.5 mole equivalents) dissolved in water ($15cm^3$). The mixture was added dropwise over a period of several minutes with continuous stirring. The solution was stirred for a further hour at room temperature during which time an orange oil separated out. The mixture was extracted with chloroform ($5x20cm^3$) and the combined organic extracts were thoroughly washed with water, dried over magnesium sulphate, filtered and evaporated under reduced pressure to give an orange oil.(0.6g, 47%). This reaction was repeated a number of times.

I.R. νmax 2225 (C≡N) and 1700 ($NCOCH_3$) cm^{-1}.

e. Preparation of 4-cyano-3-ethylpyridine (248):

The dihydropyridine (5g) from the previous reaction was dissolved in ethanol ($40cm^3$) and stirred under a medium pressure ultra violet source for thirty minutes. The solvent was removed under reduced pressure to give an orange oil which was chromatographed on a column of basic alumina using dichloromethane as eluent. The first fractions were combined, dried over magnesium sulphate, filtered and the solvent evaporated under reduced pressure to give the title compound as a colourless oil. (2.6g, 82%).

I.R. ν max 2225 (C≡N) cm^{-1},

U.V. λ max 225 and 280nm.

f. Preparation of 1-amino-3-ethylpyridinium chloride (265):

3-Ethylpyridine (16g) was added to a freshly prepared solution of hydroxylamine-O-sulphonic acid (5.7g, 0.3 mole equivalents) in cold water ($30cm^3$). The mixture was heated at about 90° on a steam bath for twenty minutes and then allowed to cool to room temperature with stirring. Potassium carbonate (6.9g) was added

and the mixture was filtered. The filtrate was thoroughly extracted with ether (5x25cm^3) to remove unreacted 3-ethylpyridine. The aqueous layer was evaporated under reduced pressure using a low temperature bath (30-40°). Absolute ethanol (170cm^3) was added to the residue and the resulting suspension was filtered. Hydrochloric acid (15cm^3, 36%) was cautiously added to the filtrate which changed from deep red to orange in colour. The solution was then evaporated to dryness to give the crude chloride as a green oil which crystallised on standing (5.7g, 59.3%).

g. Reaction between 1-amino-3-ethylpyridinium chloride (265) and dehydroacetic acid (266):

The crude chloride (265) (4.2g) and dehydroacetic acid (266) (5.1g) were added to hydrochloric acid (15cm^3,36%) and the mixture was refluxed for three hours. When cool, the mixture was stirred with charcoal (1g), and filtered to give a pale yellow solution which was evaporated to dryness. The product was triturated with dry acetone (4x50cm^3) to give the refined chloride which was dissolved in hydrochloric acid (10cm^3, 36%). The solution was filtered and poured into dry acetone (500cm^3) whereupon a fine precipitate was formed which was stirred overnight. The suspension was filtered at the pump and washed with dry acetone to give 2,6-dimethyl-1-(3-ethyl) pyridinio-4-pyridone, as the chloride hydrochloride (267). A saturated solution of the double salt was prepared in absolute ethanol, and to this was added tetrafluoroboric acid (10cm^3,40%). The mixture was stirred for half an hour and then the corresponding tetrafluoroborate salt (268) was filtered off and washed with a little absolute ethanol (3.3g,39%).

^{1}H N.M.R. δ(d^6-DMSO) 9.64 (1H,s,H-2)
9.60 (1H,d,J=6HZ,H-6),
8.99 (1H,d,J=5HZ,H-4),
8.50 (1H,m,H-5),
7.04 (2H,s,aromatic pyridone protons),
2.98 (2H,q,J=7HZ,-CH_2-CH_3),
2.22 (6H,s,ArCH_3)
and 1.33 (3H,t,J=7HZ,-CH_2-CH_3)ppm,

I.R. νmax 1630 (C=O)cm^{-1}

Reaction between 2,6-dimethyl-1-(3-ethyl) pyridinio-4-pyridone tetrafluoroborate (268) and potassium cyanide:

The pyridone (268) (2.5g) was dissolved in distilled water ($28cm^3$) and a solution of potassium cyanide (0.7g) in water ($10cm^3$) was added in a single portion with stirring. Almost immediately a precipitate was formed. Stirring was continued for fifteen minutes and then the product was extracted with chloroform ($3x20cm^3$). The combined extracts were dried over magnesium sulphate, filtered and the solvent removed under reduced pressure to give a pale yellow oil which slowly crystallised on standing (1.86g,92%).

Analysis of this material by TLC showed that it contained two major components and it was speculated that spontaneous disproportionation and aromatisation had occurred to give a mixture of 4-cyano-3-ethylpyridine (248) and 2,6-dimethyl-4-pyridone.

^{1}H N.M.R. δ($CDCl_3$) 8.65 (1H,s,H-2),
8.57 (1H,d,J=5HZ,H-6),
8.50-6.80 (1H,bs,NH from pyridone),
7.43 (1H,d,J=5HZ,H-5),
6.33 (2H,s,aromatic pyridone protons),
2.89 (2H,q,J=7HZ,-CH_2-CH_3),
2.39 (6H,s,ArCH_3),
and 1.33 (3H,t,J=7HZ,-CH_2-CH_3) ppm,

I.R. νmax 2230 (C≡N) and 1630 (ArC=O)cm^{-1}

To effect separation of the desired 4-cyano-3-ethylpyridine (248), the product from the above reaction was distilled under vacuum to give a colourless oil (0.64g,66%).

^{1}H N.M.R. $\delta(CDCl_3)$ 8.65 (1H,s,H-2),
8.58 (1H,d,J=5HZ,H-6),
7.47 (1H,d,J=5HZ,H-5),
2.91 (2H,q,J=7HZ,-CH_2-CH_3),
and 1.37 (3H,t,J=7HZ,-CH_2-CH_3)ppm,

I.R. νmax 2230 (C≡N) and 1590 (Ar) cm^{-1},

U.V. λmax 225 and 278nm,

b.p. 100° (12mm Hg).

Preparation of 4-acetyl-3-ethylpyridine (237):

a. From 4-cyano-3-ethylpyridine (248) and methyllithium:

The cyanide compound (248)(2g) was dissolved in dry ether (50cm^3) and added dropwise to a stirred solution of methyllithium in ether (14cm^3,1.38M, 1.2 mole equivalents) at 0°. After the completion of addition the mixture was stirred for a further hour and then a solution of ammonium chloride (5g) in water (20cm^3) was added cautiously to decompose the excess organometallic reagent. The mixture was extracted with chloroform (3x20cm^3) and the combined extracts were dried over magnesium sulphate, filtered and the solvent evaporated under reduced pressure to give the desired imine (249) as a colourless oil (1.5g, 64%).

^{1}H N.M.R. $\delta(CDCl_3)$ 8.47 (1H,s,H-2),
8.43 (1H,d,J=5HZ,H-6),
7.14 (1H,d,J=5HZ,H-5),
3.41 (2H,q,J=7HZ,-CH_2-CH_3),
2.33 (3H,s,-CH_3),
and 1.24 (3H,t,J=7HZ,-CH_2-CH_3)ppm,

I.R. νmax 3270 (N-H) and 1640 (C=N)cm^{-1}.

The imine (249) (1.5g) was dispersed in acetic acid ($20cm^3$,60%) and stirred for thirty minutes at room temperature. The mixture was then cooled in an ice bath and made basic with sodium carbonate before extraction with chloroform ($3x50cm^3$). The combined extracts were dried over magnesium sulphate, filtered and the solvent removed under reduced pressure to give a dark orange oil. Distillation of this material under vacuum gave the title compound as a colourless liquid (0.85g,56%).

^{1}H N.M.R. $\delta(CDCl_3)$ 8.60 (1H,s,H-2),
8.55 (1H,d,J=5HZ,H-6),
7.31 (1H,d,J=5HZ,H-5),
2.80 (2H,q,J=7HZ,-CH_2-CH_3),
2.58 (3H,s,-$COCH_3$),
and 1.20 (3H,t,J=7HZ,-CH_2-CH_3)ppm,

I.R. νmax 1700 (C=O)cm^{-1},

U.V. λmax 225 and 278nm,

M.S. m/e (rel.int.%) 149(M^+,78) and 134(100),

b.p. 92° (0.3mm Hg).

b. From 3-ethylpyridine and acetic anhydride:

3-Ethylpyridine (20g) was dissolved in acetic anhydride ($300cm^3$) and the mixture was cooled in an ice-salt bath with continuous stirring. Zinc dust (20g) was added portionwise to the stirred solution over a period of three hours. The mixture was stirred at the depressed temperature for several hours and was allowed to warm up to room temperature overnight. The remaining zinc and zinc acetate were removed by filtration and the excess acetic anhydride was evaporated under reduced pressure at 40° to leave a yellow oil. The residual

oil was distilled under vacuum as a single fraction boiling at 40° (0.15 mm Hg).

^{1}H N.M.R. δ($CDCl_3$) 14.20 (1H,s,N<u>H</u>),
8.50 (2H,dd,<u>J</u>=2HZ and <u>J</u>=5HZ, H-2 and H-6),
7.63 (1H,dt,<u>J</u>=2HZ and <u>J</u>=8HZ, H-4),
7.27 (1H,dd,<u>J</u>=5HZ and <u>J</u>=8HZ, H-5),
2.66 (2H,q,<u>J</u>=7HZ,-C<u>H</u>$_2$-CH_3),
2.08 (3H,s,COC<u>H</u>$_3$)
and 1.24 (3H,t,<u>J</u>=7HZ,-CH_2-C<u>H</u>$_3$)ppm

The oil from the above reaction (4.0g) was dissolved in glacial acetic acid (40cm^3) and the stirred mixture was treated dropwise with a solution of chromium trioxide (0.8g) in water (10cm^3) over a period of fifteen minutes. The mixture was stirred for an hour at room temperature and then propan-2-ol (10cm^3) was added and stirring continued for a further fifteen minutes. The solvents were removed at 35° under vacuum to leave a brown gum which was extracted with saturated sodium hydrogen carbonate solution (100cm^3) and ether (100cm^3). The organic phase was separated, washed with brine (50cm^3) and re-extracted with hydrochloric acid (50cm^3,1M). The acid layer was washed with ether (2x50cm^3) and then made basic with solid sodium hydrogen carbonate. The product was then re-extracted with ether (3x50cm^3). The ether extracts were bulked, dried over sodium sulphate, filtered and the solvent removed under reduced pressure to give a pale yellow oil. Spectroscopic analysis of this material showed it to be unreacted 3-ethylpyridine.

^{1}H N.M.R. δ($CDCl_3$) 8.35 (2H,dd,<u>J</u>=2HZ and <u>J</u>=5HZ, H-2 and H-6),
7.50 (1H,dt,<u>J</u>=2HZ and <u>J</u>=8HZ,H-4),
7.16 (1H,dd,<u>J</u>=5HZ and <u>J</u>=8HZ,H-5),
2.63 (2H,q,<u>J</u>=7HZ,-C<u>H</u>$_2$-CH_3),
and 1.25 (3H,t,<u>J</u>=7HZ,-CH_2-C<u>H</u>$_3$) ppm,

Preparation of ethylacetimidate hydrochloride (254):

Dry acetonitrile (79cm^3, 106g), absolute ethanol (119cm^3, 1 mole equivalent) and anhydrous ether (170cm^3,0.5mole equivalents) were stirred together in an ice-salt bath while dry hydrogen chloride gas was bubbled through the mixture over a period of about four hours. After one molecular equivalent of hydrogen chloride had been absorbed, corresponding to a weight increase of 94g, the mixture was left to stand until crystallisation occurred. The remaining solvent was decanted and evaporated to dryness, giving an additional crop of white crystals which was combined with the rest. The product was collected at the pump, washed with anhydrous ether and dried over potassium hydroxide and phosphorus pentoxide in a vacuum desiccator (262g,82%).

^{1}H N.M.R. δ(d^6-DMSO) 11.50 (2H,bs,$^{\oplus}NH_2$),
4.42 (2H,q,J=7HZ,-CH_2-CH_3),
2.31 (3H,s,-CH_3),
and 1.20 (3H,t,J=7HZ,-CH_2-CH_3)ppm,

I.R. νmax 1640 (C=NH) cm^{-1},

m.p. 300°

Preparation of 1-ethoxy-1-oximidoethane (255):

Ethyl acetimidate hydrochloride (254)(50g) was added portionwise to a solution of potassium carbonate (112g,2 mole equivalents) in water (250cm^3) chilled in an ice bath. The mixture was then vigorously stirred for fifteen minutes at room temperature, during which time a clear colourless oil separated out. The aqueous phase was removed and extracted with ether (3x100cm^3). The combined extracts and the product layer were then cooled in an ice-salt bath while a solution of hydroxylamine hydrochloride (30g,1.25 mole

equivalents) in water (125cm^3) was quickly added. The mixture was stirred vigorously for half an hour and then the ether layer was separated. The aqueous phase was extracted with ether (4x100cm^3) and the combined ether portions were dried over sodium sulphate. The solution was filtered and the solvent removed under reduced pressure to give the product as a colourless oil. On cooling the oil, long white needles were obtained (30.6g,73%).

I.R. νmax 3600-3200 (O-H, associated), 1665(C=N) and 1300 (O-H, deformation)cm^{-1},

m.p. 19°.

Preparation of mesitylenesulphonyl chloride (256):

Chlorosulphonic acid (167cm^3, 3 mole equivalents) was cooled in an ice bath and mesitylene (116 cm^3, 1 mole equivalent) was added dropwise over a period of three hours with stirring. After the completion of the addition the mixture was stirred for a further hour at room temperature, and then poured onto crushed ice. The semi-solid compound was extracted with tetrachloromethane (500cm^3), washed with sodium carbonate solution (3x200cm^3, 2N) and dried over magnesium sulphate. The solution was filtered and the solvent evaporated under reduced pressure to give an oil which slowly crystallised on standing. Recrystallisation from 40-60° boiling fraction petroleum ether gave the title compound as white prisms (169.4g, 93%).

I.R. νmax 1600,1580(Ar), 1360(SO_2), 1180 and 1170 (SO_2)cm^{-1},

m.p. 50-52°

Preparation of ethyl-O-(mesitylenesulphonyl) acetohydroxamate (257):

1-Ethoxy-1-oximidoethane (255) (20g) was dissolved in N,N-dimethyl-

formamide ($100cm^3$) and triethylamine ($30cm^3$, 1.1 mole equivalents) and the mixture was immersed in a bath of cold water. Mesitylene-sulphonyl chloride (256) (42.4g, 1 mole equivalent) was then added portionwise to the stirred mixture over a period of forty-five minutes. The mixture was stirred for an additional hour after the completion of the addition, and was then poured onto crushed ice (500g). On standing for a few minutes, a pale straw-coloured solid was precipitated which was collected at the pump,washed with 60-80° boiling fraction petroleum ether and sucked dry. The product was stored in the refrigerator until required to prevent hydrolysis to ethyl acetate (44.2g, 79%).

I.R. νmax 1645 (C=N), 1360 (SO_2O, asy.) and 1180 (SO_2O,sy.)cm^{-1},

m.p. 48-50° dec.

Mesitylenesulphonyl hydroxylamine (258):

The precursor (257) (20g) was added to perchloric acid ($70cm^3$, 60%) and the mixture warmed to 35° for two minutes to initiate reaction. The mixture was then stirred for twenty minutes at room temperature, during which time all of the solid material dissolved to give a brown solution. This was poured into iced water ($800cm^3$) and a white solid precipitated which was allowed to stand for five minutes. The solid was quickly filtered, washed with a chilled solution of sodium hydrogen carbonate ($125cm^3$,1M) and then washed again with iced water ($200cm^3$). The solid was dissolved in ether and dried over sodium sulphate. The solution was filtered and the solvent was removed under reduced pressure in a cold water bath to prevent thermal decomposition. The product was obtained as a white solid which was stored in the refrigerator until required (12.1g,92%).

I.R. νmax 3270 and 3230 (NH_2), 1365 and 1170 (SO_2O)cm^{-1}

Preparation of 2-methyl-3-nitroaniline (272):

Powdered 2,6-dinitrotoluene (25g) was placed in a $1dm^3$ round-bottom, three-neck flask, together with distilled water ($100cm^3$) and the mixture was heated almost to boiling with continuous stirring. Sodium sulphide (40g) and powdered sulphur (10g) were dissolved in water ($150cm^3$) with warming to give a red-brown solution of sodium polysulphide which was added dropwise to the vigorously stirred reaction mixture over a period of about forty-five minutes. Heating was maintained for a further twenty minutes, and then the reaction mixture was allowed to cool before filtering at the pump. The solid residue was washed with a little cold water and then transferred to a $600cm^3$ beaker containing water ($150cm^3$) and hydrochloric acid ($35cm^3$,36%). The mixture was brought to boiling for fifteen minutes, during which time the amine dissolved, leaving sulphur and unchanged 2,6-dinitrotoluene as suspended solids. The mixture was filtered and the filtrate treated with concentrated ammonia solution to give the product as a bright yellow precipitate which was collected at the pump. A second crop was obtained by repeating the extraction procedure on the sulphurous residue. Recrystallisation from ethanol gave the title compound as yellow needles (14.2g, 68%).

^{1}H N.M.R. $\delta(CDCl_3)$ 7.20-6.66 (3H, complex, H-3,H-4 and H-5),
3.83 (2H,bs,-$\underline{NH_2}$),
and 2.14 (3H,s,-$\underline{CH_3}$)ppm,

I.R. νmax 3400, 3320 (N-H), 3200 (N-H) and 1600 (Ar)cm^{-1},

U.V. λmax 208,239 and 292 (sh)nm,

m.p. 84°

Preparation of 2-hydroxy-6-nitrotoluene (273):

2-Amino-6-nitrotoluene (272)(18g), concentrated sulphuric acid ($30cm^3$) and water ($50cm^3$) were brought to boiling in a $600cm^3$ beaker.

The resulting solution was rapidly chilled which precipitated the amine as a finely divided suspension. Crushed ice (50g) was added to the mixture which was cooled in an ice-salt bath. A solution of sodium nitrite (7.5g) in water ($20cm^3$) was added slowly to the stirred mixture over a period of one hour. Stirring was continued for a further hour at the depressed temperature and then urea (10g) was added to decompose excess sodium nitrite. The mixture was filtered and the filtrate added slowly to a boiling solution of concentrated sulphuric acid ($70cm^3$) and water ($50cm^3$). After addition was complete the mixture was stirred at reflux temperature for three hours, and then allowed to cool. The product precipitated out as yellow needles which were collected at the pump, washed with a little 60-80 boiling fraction petroleum ether and dried in a desiccator over phosphorus pentoxide (12.9g, 71%).

^{1}H N.M.R. δ (d^6-DMSO) 7.45-7.11 (3H,complex, H-3,H-4 and H-5),
6.60-5.10 (1H,bs,-O$\underline{H}$),
and 2.23 (3H,s,-C$\underline{H}_3$) ppm,

I.R. ν max 3400 (b,O-H) and 1600 (Ar)cm^{-1}

U.V. λ max 220,244,273 and 335nm,

m.p.

Preparation of 2-benzyloxy-6-nitrotoluene (274):

A solution of 2-hydroxy-6-nitrotoluene (273) (20g) in ethanol ($120cm^3$) was added to a solution of sodium hydroxide (5.5g) in water ($40cm^3$). Benzyl chloride (15.5g) was added and the mixture was allowed to reflux gently overnight for sixteen hours. The solvent was evaporated under reduced pressure to give an oil which solidified on standing. The product was recrystallised from a 1:1 mixture of benzene and 80-100° boiling fraction petroleum ether (24g,76%).

^{1}H N.M.R. $\delta(CDCl_3)$ 7.41 (5H,s,H-2' to H-6' inclusive),
7.33-6.97 (3H,complex, H-3, H-4 and H-5),
5.09 (2H,s,-$\underline{CH_2}$-),
and 2.37 (3H,s,-$\underline{CH_3}$) ppm,

I.R. νmax 1600 (Ar)cm^{-1},

U.V. λmax 219, 243, 273 and 335nm,

M.S. m/e (rel.int.%) 243(M^+), 136(100), 108(35) and 77(81),

m.p. 48-52°

Attempted preparation of 2-benzyloxy-6-nitrobenzaldiacetate (275):

Glacial acetic acid (130cm^3), acetic anhydride (130cm^3) and 2-benzyloxy-6-nitrotoluene (274)(20g) were stirred together in a chilled vessel. Concentrated sulphuric acid (20cm^3) was added slowly to the mixture, ensuring that there was no sudden increase in temperature. When the mixture had cooled to below 5°, chromium trioxide (23g) was added in small portions over a period of ninety minutes, taking care that the temperature did not exceed 10°. After completion of the addition stirring was continued for a further fifteen minutes and then the contents of the flask were poured onto crushed ice (500g) and the mixture was diluted to about 1300cm^3 with chilled water. The mixture was then filtered through a glass sinter, but no solid remained. A portion of the green aqueous phase was extracted with chloroform, and the combined extracts were dried over magnesium sulphate, filtered and the solvent removed under reduced pressure to give an orange oil which could not be characterised.

Attempted preparation of 2,6-dinitrobenzaldiacetate:

1. Powered 2,6-dinitrotoluene (3.6g), glacial acetic acid (33cm^3) and acetic anhydride (33cm^3) were stirred together and cooled in an

ice-salt bath. Concentrated sulphuric acid ($4cm^3$) was added dropwise to avoid charring and the mixture was cooled to below 5°. Chromium trioxide (5.7g) was added in small portions over a period of one hour, and at all times the temperature was maintained below 10°. Stirring was continued for a further thirty minutes after the addition was complete and then the contents of the flask were poured onto crushed ice (100g). Cold water was added to make the total volume of the mixture up to $350cm^3$. The solid was separated by suction filtration and washed with cold water until the washings were colourless.

The product was suspended in cold sodium carbonate solution ($30cm^3$, 2%) and stirred. After thorough mixing, the solid was collected at the pump and washed with cold water and a little cold alcohol (2.9g, 80%).

Analysis of this material by TLC showed it to be unchanged starting material, a result which was confirmed by its infra red spectrum which could be superimposed upon that of unreacted 2,6-dinitrotoluene.

2. When chromium trioxide was added to the reaction mixture at room temperature the same result was achieved.

Preparation of benzyl-tri-n-butylammonium bromide:

Freshly distilled tri-n-butylamine ($48cm^3$) was added to benzyl bromide ($38cm^3$) and dry acetonitrile ($60cm^3$). The mixture was gently refluxed for one week and then left to cool, resulting in a deep brown coloured solution. Most of the acetonitrile was evaporated under reduced pressure and a pale brown precipitate was obtained. Anhydrous ether ($50cm^3$) was added to precipitate more of the solid which was collected by suction filtration. Recrystallisation

from ethyl acetate containing a little ethanol yielded the product as large colourless prisms (60g,82%).

Preparation of 2-methoxy-6-nitrotoluene (278):

2-Hydroxy-6-nitrotoluene (273)(3.1g, 0.02 mole) was dissolved in dichloromethane ($100cm^3$) and dimethyl sulphate ($5cm^3$, 0.05 mole) was added. Sodium hydroxide (1.2g, 0.03 mole) and benzyl-tri-n-butylammonium bromide (0.7g, 0.002 mole) were dissolved in cold water ($100cm^3$) and the two solutions were combined. The mixture was then shaken overnight at room temperature. The organic layer was separated and the aqueous phase was extracted with dichloromethane ($2x50cm^3$). The combined organic extracts were evaporated under reduced pressure and the residue was mixed with water ($50cm^3$) and exhaustively extracted with 30-40° boiling fraction petroleum ether. The organic extract was then washed with dilute ammonia solution ($100cm^3$) to remove excess dimethyl sulphate, followed by sodium hydroxide solution ($100cm^3$, 2M) to remove unreacted phenol. After drying over magnesium sulphate and filtration, the solvent was evaporated under reduced pressure to give the methyl ether (271) as pale yellow needles (3.1g,93%).

^1H N.M.R. δ (d^6-DMSO) 7.60-7.24 (3H, complex,H-3,H-4 and H-5), 4.03 (3H,s,-O-CH_3) and 2.39 (3H,s,-CH_3)ppm,

I.R. ν max 1600 (Ar) cm^{-1},

U.V. λ max 219, 244, 270 and 330nm,

m.p. 48-51° (lit.[132] 51-53°).

Preparation of 2-methoxy-6-nitrobenzaldiacetate (279):

2-Methoxy-6-nitrotoluene (278)(3.34g, 0.02 mole), glacial acetic acid ($33cm^3$, 0.6 mole) and acetic anhydride ($33cm^3$,0.25 mole)

were cooled and thoroughly mixed together in an ice-salt bath. Concentrated sulphuric acid ($4cm^3$) was added cautiously to the stirred mixture to avoid charring. When the temperature had returned to 0°, chromium trioxide (5.7g, 0.057 mole) was added portionwise over a period of thirty minutes, ensuring that the temperature did not exceed 10°. After the completion of the addition, stirring was continued for a further thirty minutes, and then the contents of the flask were poured onto crushed ice ($150cm^3$). Cold water was added so that the total volume was approximately $350cm^3$. The resulting solid was separated by suction filtration and washed with copious quantities of cold water until the washings were colourless. The product was suspended in sodium carbonate solution ($30cm^3$, 2%) and stirred thoroughly. The solid was then collected at the pump, washed with cold water and ice-cold ethanol ($2cm^3$) and sucked dry. Recrystallisation from ethanol gave the product as orange prisms (0.7g,12.5%).

^{1}H N.M.R. $\delta(CDCl_3)$ 8.14 (1H,s,Ar-C<u>H</u>-$(OCOCH_3)_2$),
7.66-7.00 (3H, complex, H-3, H-4 and H-5),
3.94 (3H,s,-O-C<u>H</u>$_3$)
and 2.08 (6H,s,-COC<u>H</u>$_3$)ppm,

I.R. νmax 1760 (C=O)and 1600 (Ar)cm^{-1}

Preparation of 2-methoxy-6-nitrobenzaldehyde (280):

2-Methoxy-6-nitrobenzaldiacetate (279)(0.7g) was dissolved in a mixture of ethanol ($10cm^3$) and water ($10cm^3$) in a $50cm^3$ round bottom flask. Concentrated sulphuric acid ($2cm^3$) was added and the mixture was refluxed for about thirty minutes. The cooled reaction mixture was filtered through a fluted filter paper to remove insoluble impurities and the filtrate was chilled in an ice bath to precipitate the product. The crystals were separated by suction filtration,

washed with cold water and dried in a vacuum desiccator. A second crop of crystals was obtained by further diluting the filtrate with cold water (0.45g, 63%).

^{1}H N.M.R. $\delta(CDCl_3)$ 10.44 (1H,s,-CHO),
7.80-7.18 (3H,complex, H-3, H-4 and H-5),
and 4.01 (3H,s,-OCH_3)ppm,

I.R. νmax 1710 (C=O) and 1600 (Ar) cm^{-1}

Preparation of 2-methoxy-6-nitrobenzoic acid (285):

1. From 2-methoxy-6-nitrotoluene (278) and potassium dichromate:

2-Methoxy-6-nitrotoluene (278) (5g) and potassium dichromate (22g) were suspended in water (50cm^3) and the mixture was stirred thoroughly. Concentrated sulphuric acid (35cm^3) was added dropwise with stirring and the reaction was initiated with slight warming until the solution began to show a green colouration. When the heat of reaction had subsided after about an hour, the mixture was brought slowly to the boil and refluxed gently for half an hour. The mixture was allowed to cool and water (150cm^3) was added with stirring. The precipitated product was filtered off and initial investigation of the crude material by TLC showed that it was unreacted starting material. It was insoluble in sodium carbonate solution, even on warming, which suggested that there was no carboxylic acid group present.

When this reaction was repeated and the period of reflux was extended to some sixty hours, unchanged starting material was again returned.

2. From 2-methoxy-6-nitrotoluene (278) and potassium permanganate:

2-Methoxy-6-nitrotoluene (278)(10g) and potassium permanganate (15g) were suspended in water (250cm^3) and the stirred mixture was refluxed gently until the colour of the permanganate had almost

disappeared. At this point a second portion of permanganate (7.5g) was added and heating continued for a further two hours. Finally, a third portion of permanganate (7.5g) was added and heating was maintained until its colour had been discharged. After this time the reaction mixture was allowed to cool and was then separated from the precipitated manganese dioxide by suction filtration. Unreacted starting material was extracted from the reaction mixture with 30-40° boiling fraction petroleum ether ($3x100cm^3$) and then the aqueous phase was concentrated to about $150cm^3$ by evaporation under reduced pressure. The acid (285) was precipitated by the addition of hydrochloric acid ($15cm^3$, 36%) with continuous stirring. When the mixture had cooled the solid was collected at the pump, washed with cold water and allowed to dry in air. Recrystallisation from aqueous ethanol afforded 2-methoxy-6-nitrobenzoic acid (285) as pale yellow needles (3.1g,27%). Recovery of 2-methoxy-6-nitrotoluene was 4.7g (47%).

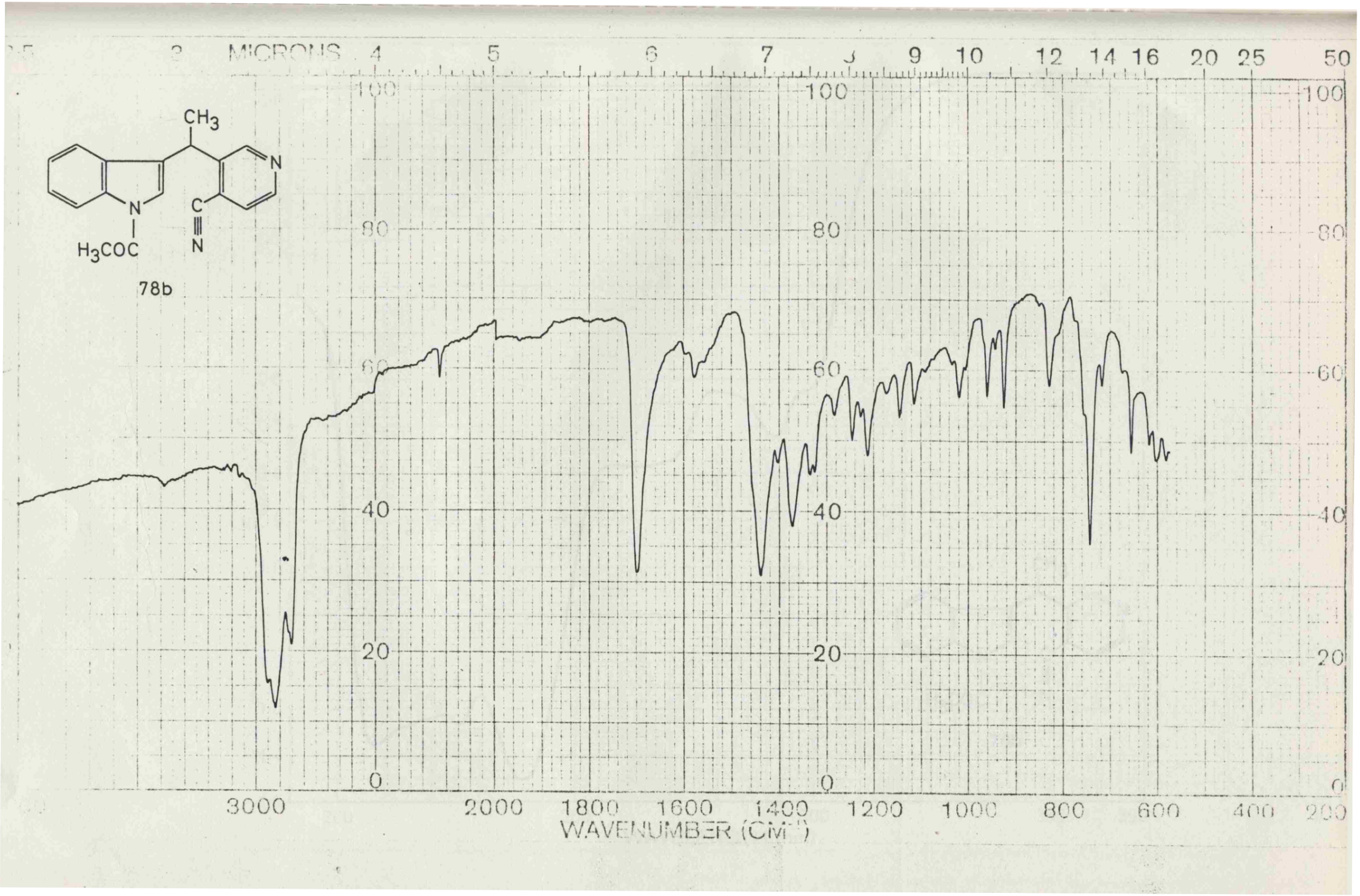
^{1}H N.M.R. $\delta(CD_3OD)$ 7.76-7.35 (3H, complex, H-3, H-4 and H-5), and 3.93 (3H,s,-OCH_3) ppm,

I.R. νmax 3300-2500 (O-H, associated), 1710 (C=O) and 1600 (Ar)cm^{-1},

U.V. λmax 217,235(sh), 272 and 333nm,

m.p. 55°.

SPECTRA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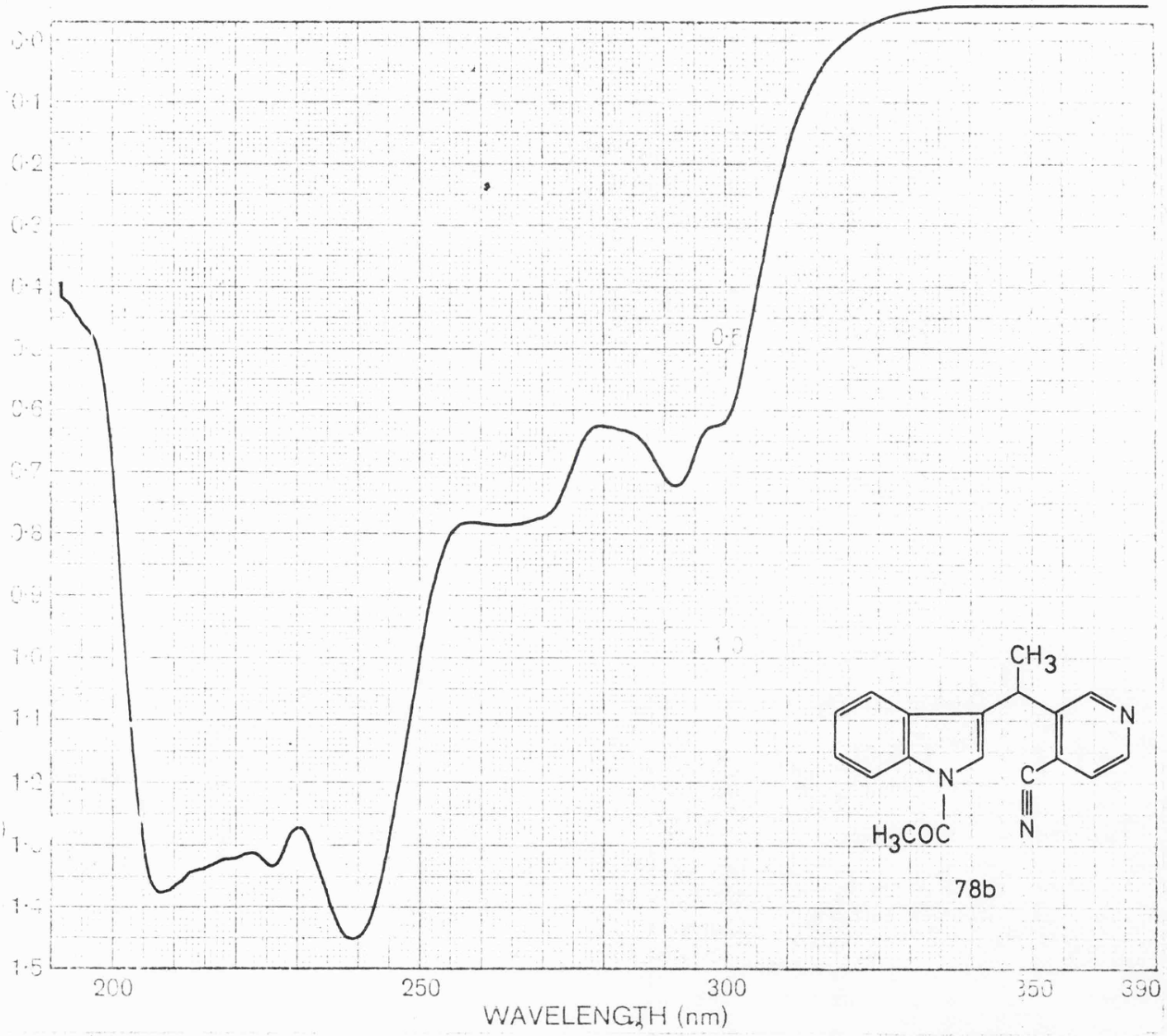
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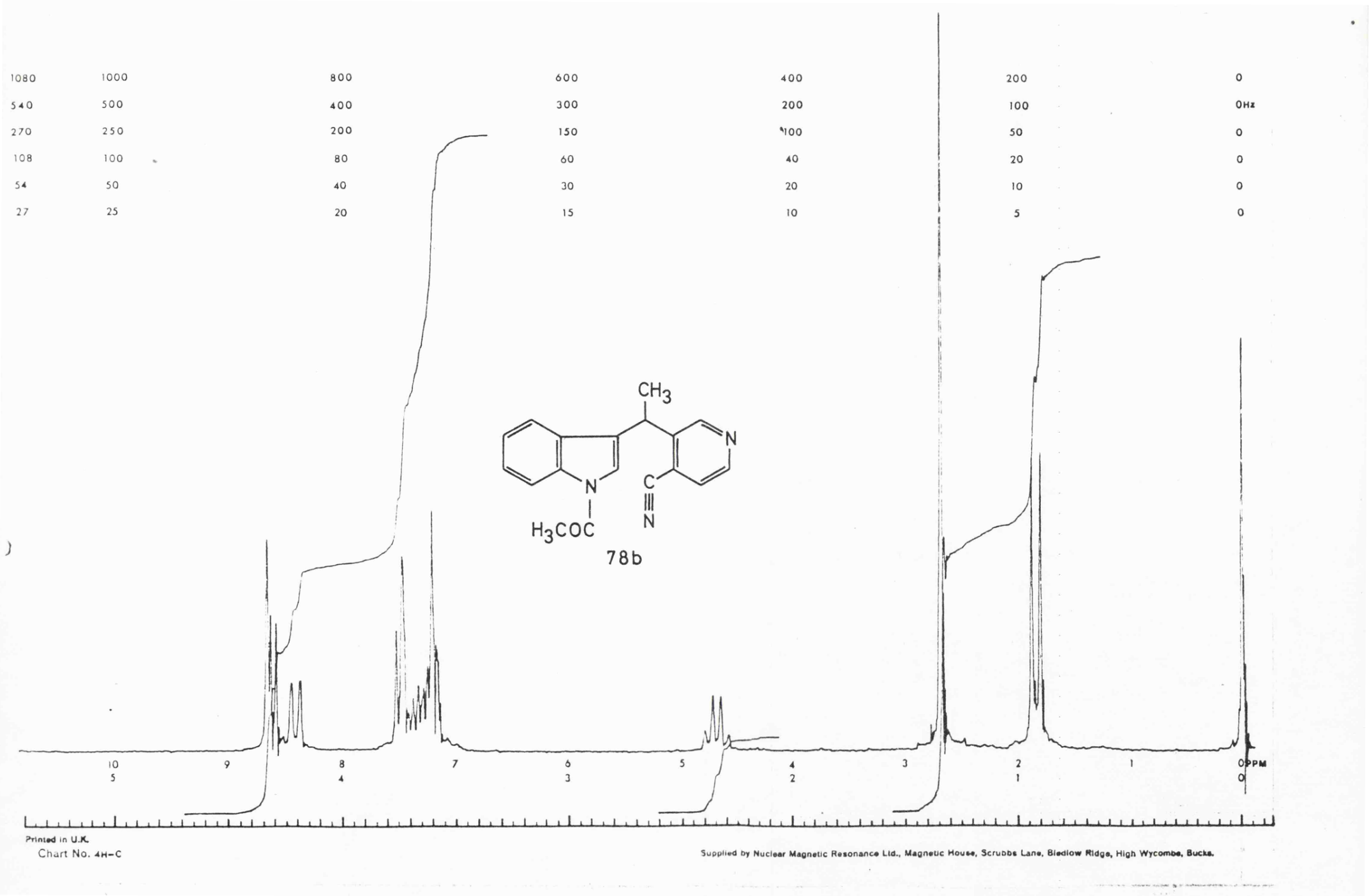

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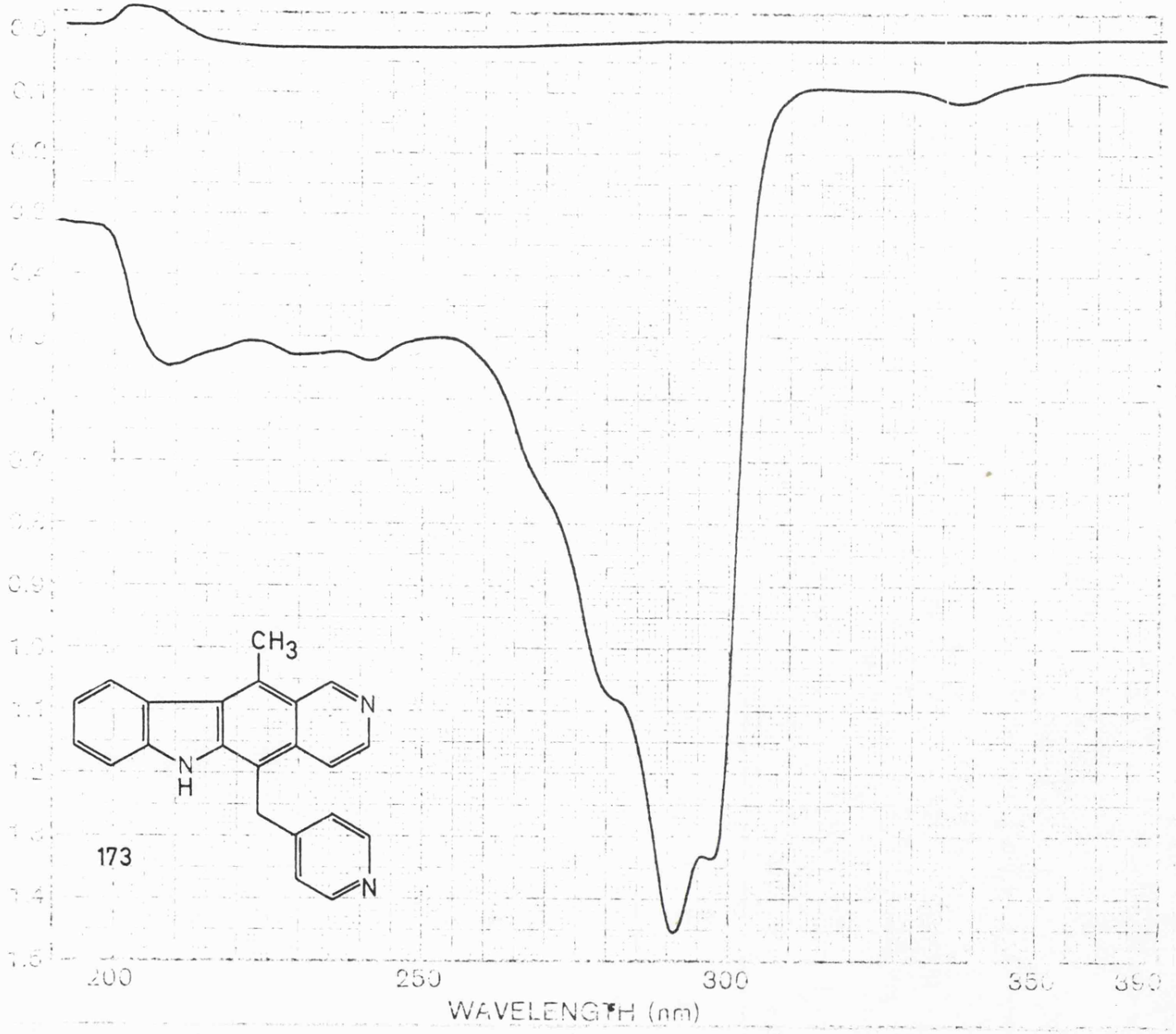
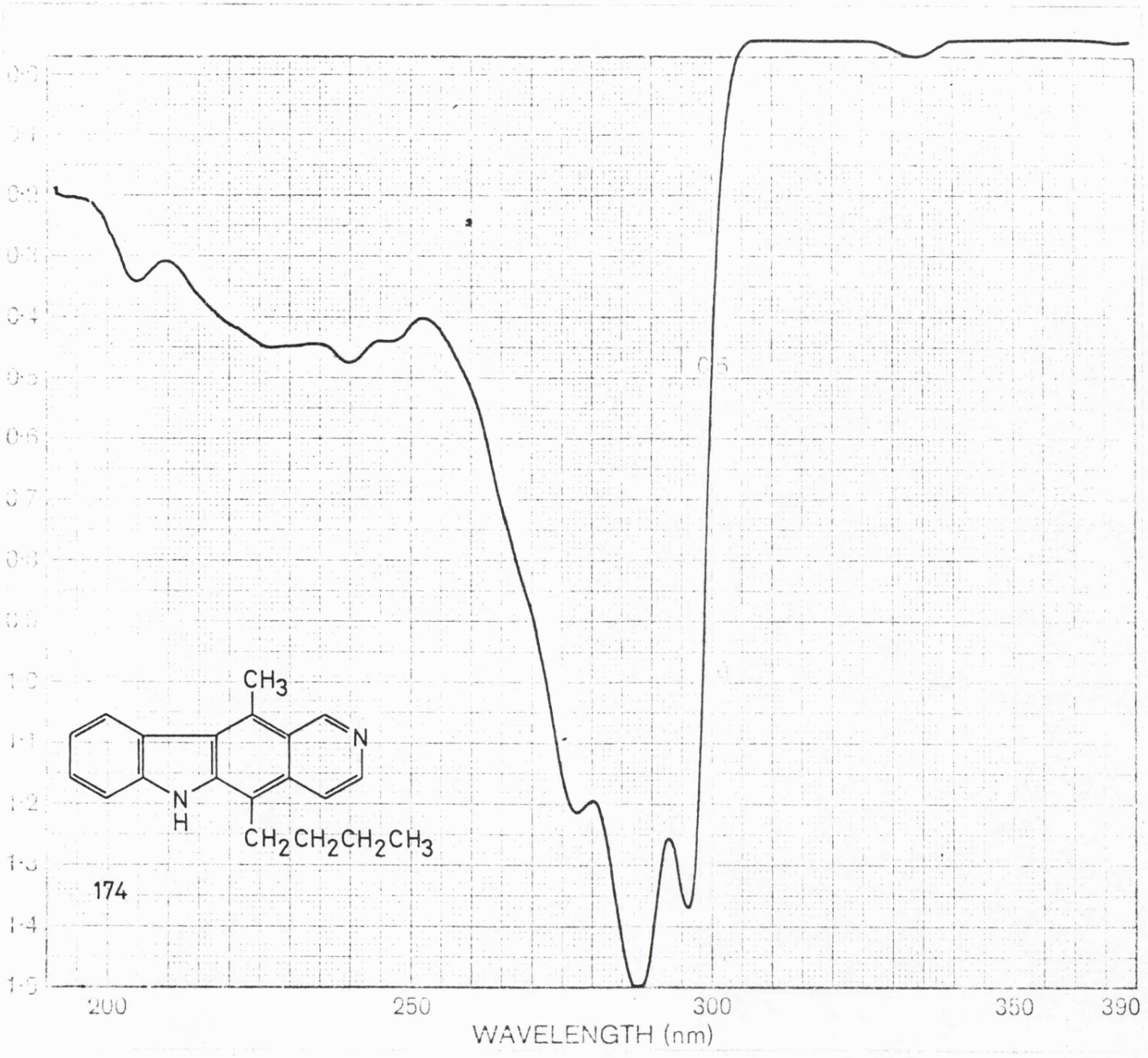
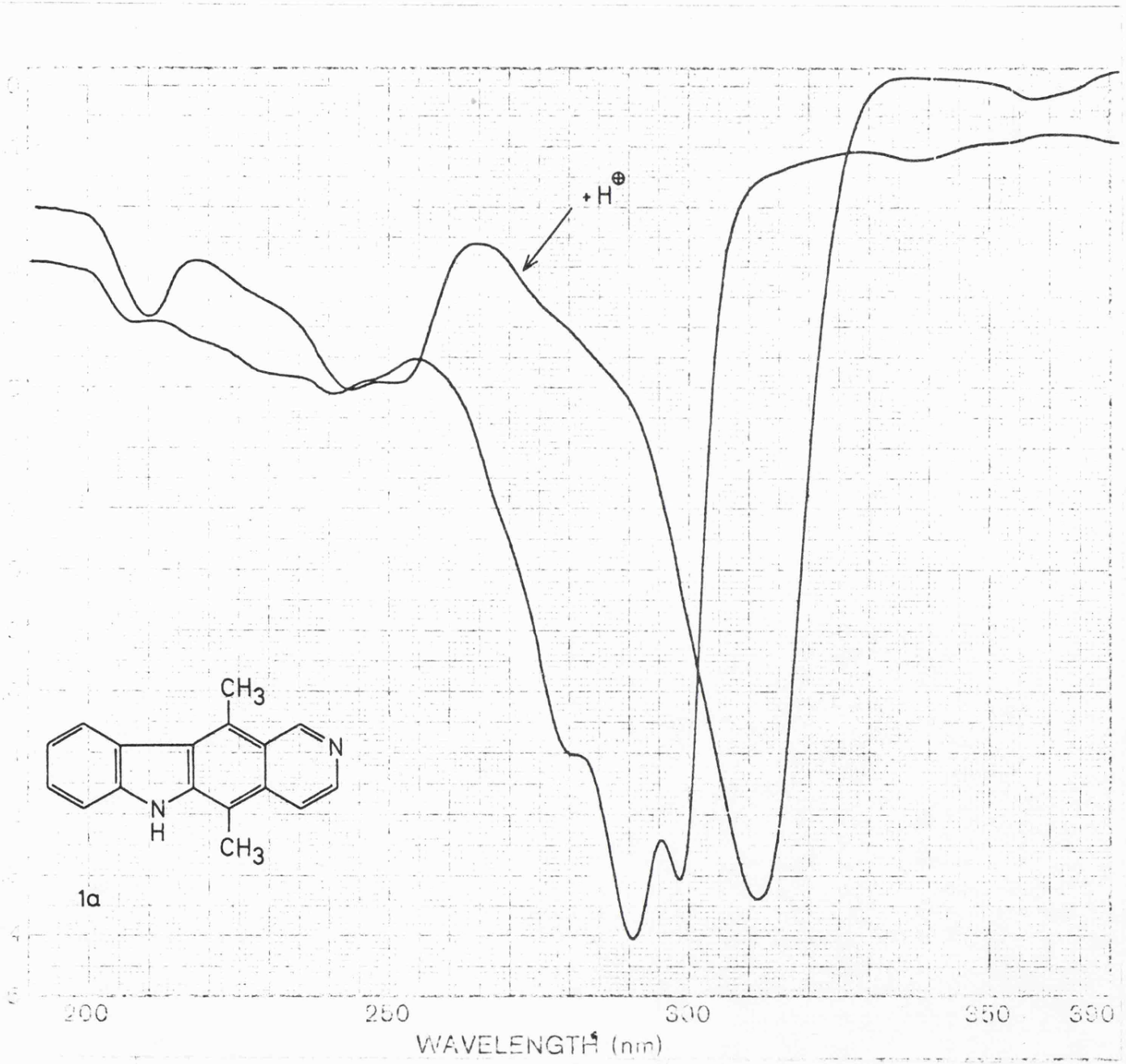
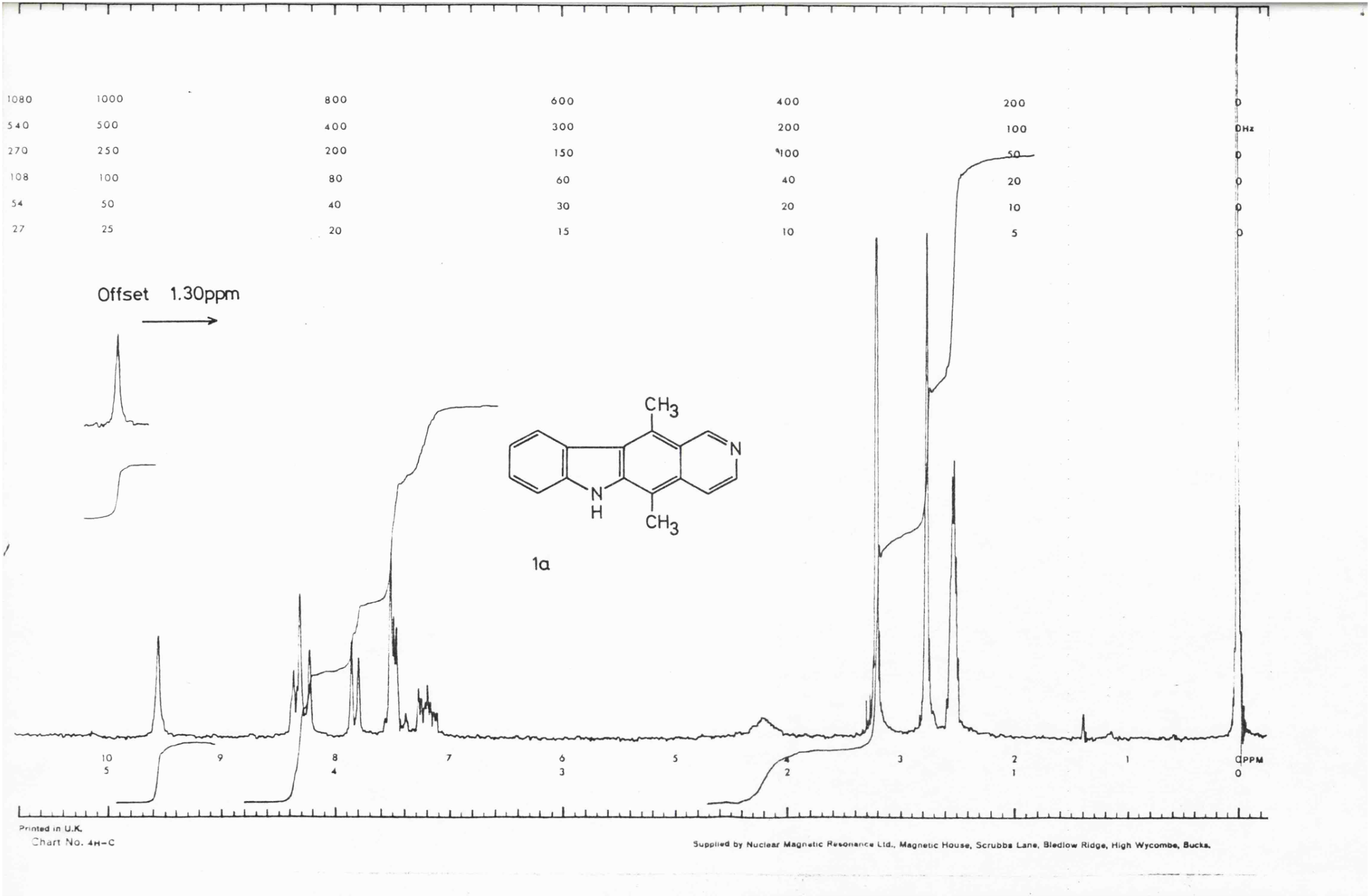
Chart No. 4H-C

Supplied by Nuclear Magnetic Resonance Ltd., Magnetic House, Scrubbs Lane, Bledlow Ridge, High Wycombe, Bucks.


CH3
N
N
H
N
173
200
250
300
WAVELENGTH (nm)


200
250
300
350
390
WAVELENGTH (nm)
CH3
N
N
H
CH2CH2CH2CH3
174


+H⊕
CH3
N
N
H
CH3
1a
200
250
300
350
400
WAVELENGTH (nm)



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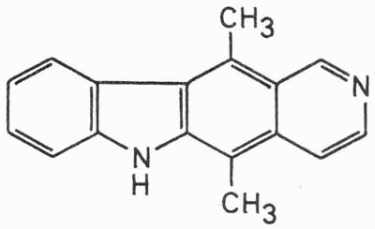
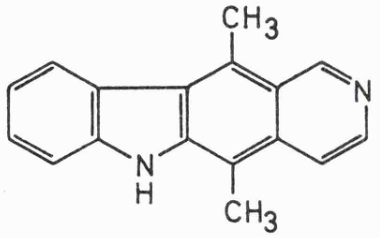
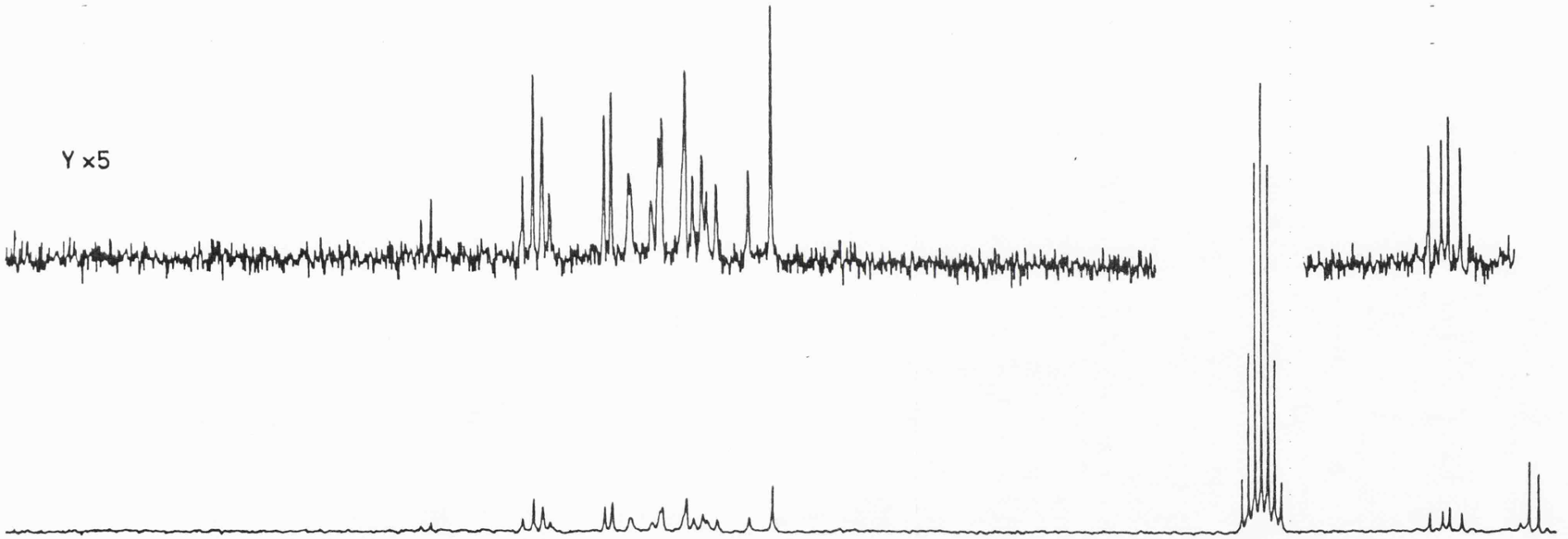


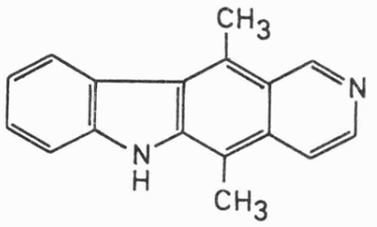
1a C-13 NMR
32,000 pulses

X ×5, Y ×10

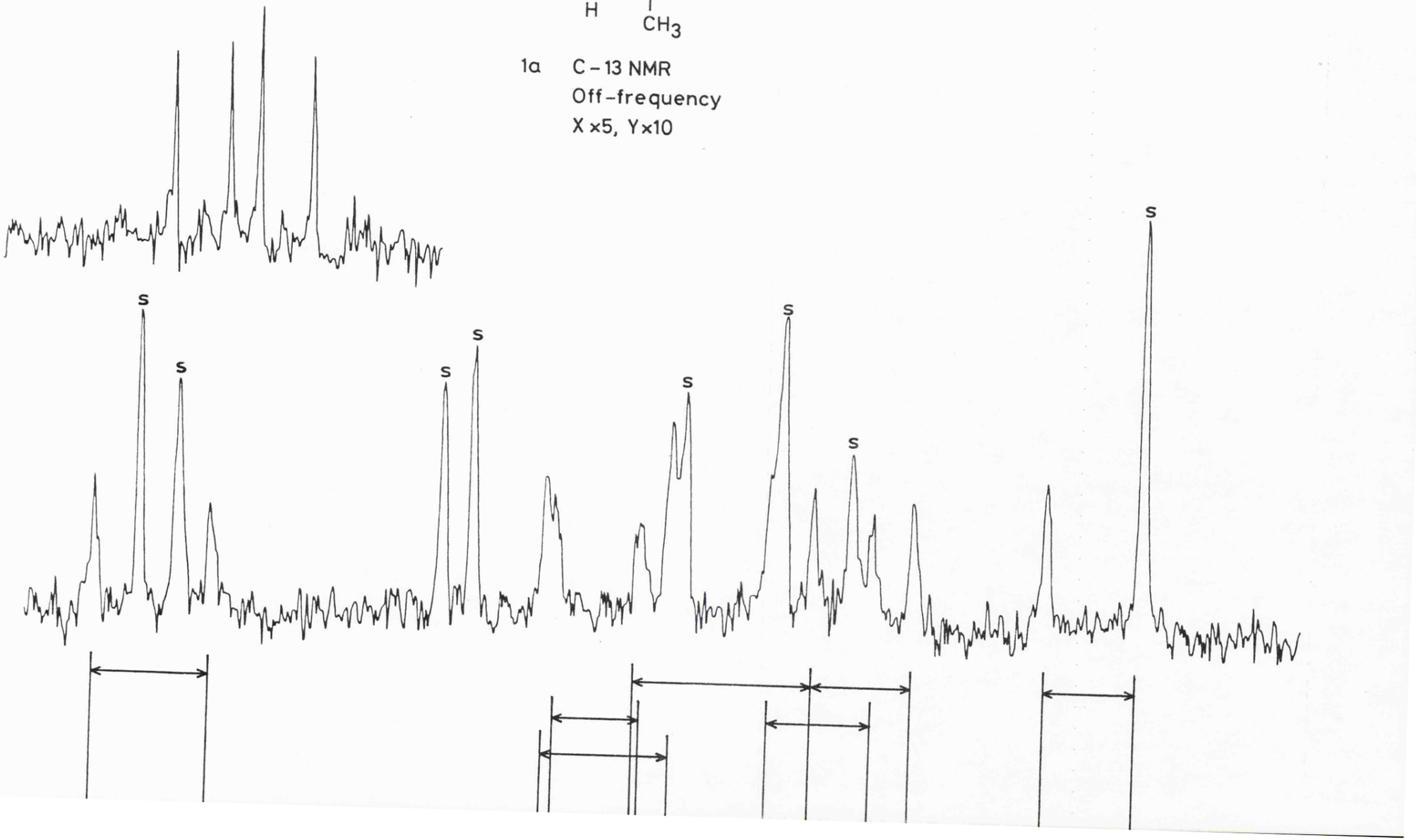


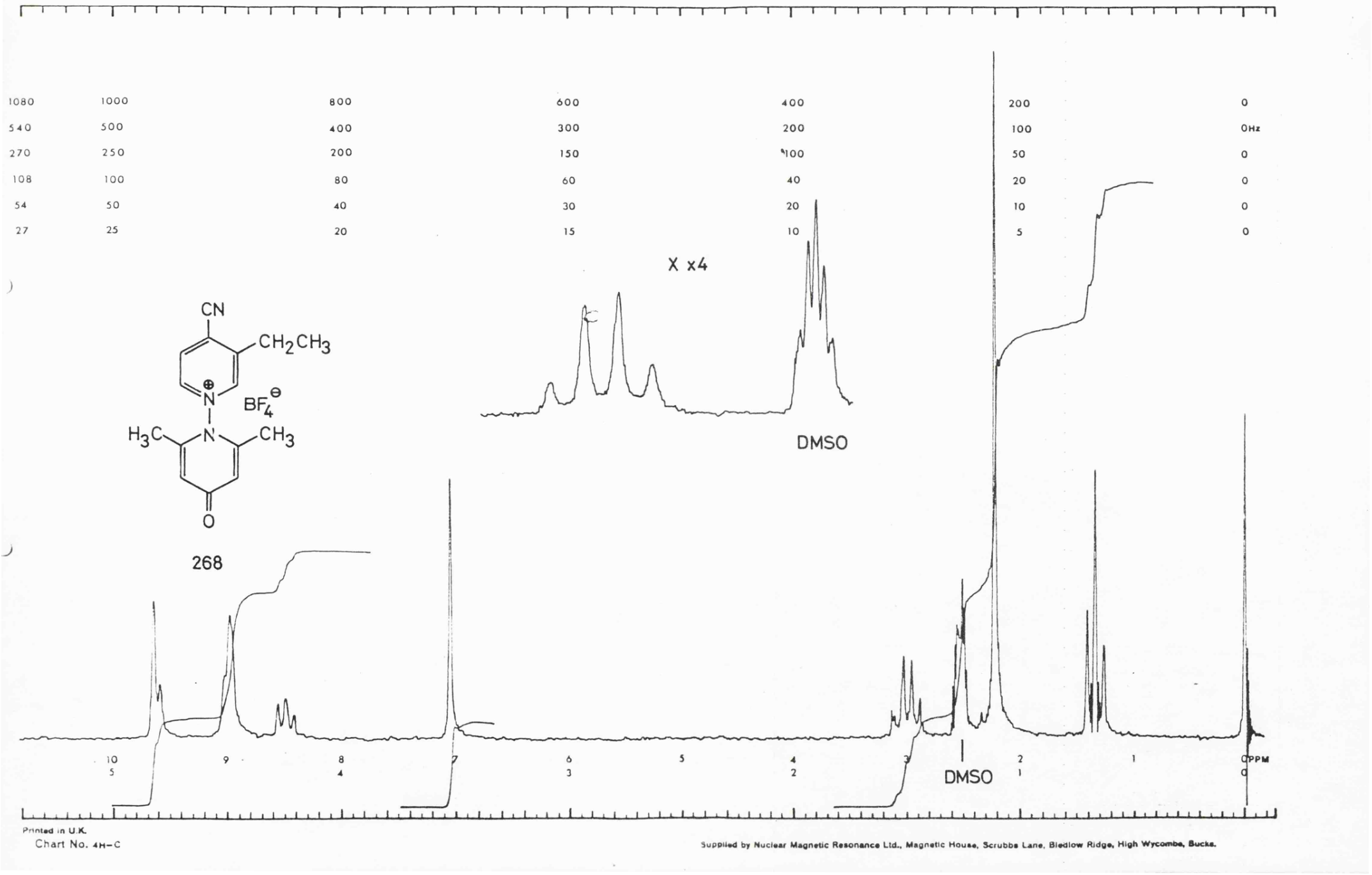
1a C-13 NMR
Off-frequency





1a C – 13 NMR
Off-frequency
X ×5, Y×10





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Unpublished results.